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Optimization of papaya latex and papain proteolytic activity and evaluation of antioxidant activities of latex

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ABSTRACT:

One of the main enzymes found in papaya is papain, which is used in many different fields. Therefore, Papain was collected, isolated, and purified from the papaya plants. This study focuses on extracting and purifying papain from papaya latex (PL) using the Three-Phase Partitioning (TPP) method. The protease activity, protein content, specific activity, activity recovery, and purification fold were measured in both the intermediate and bottom layers to compare their differences. Then, after extraction of papain enzyme from the latex, the effects of different parameters, including pH, temperature, and papain concentration, on protease activity were tested to optimize papain activity. In this respect, it was found that pH 7, 60 ºC and a concentration of 2% were the optimum parameters for protease activity of the papain enzyme. In addition, the antioxidant activity of the extracted PL from papaya fruits was evaluated using total antioxidant activity, reducing power, and DPPH assays. The antioxidant activity, reducing power, and DPPH scavenging activity of PL increased gradually with increasing PL concentration. Furthermore, the total phenolic content (TPC) and total flavonoids content (TFC) of PL were determined to confirm the potential antioxidant activity of PL. It was found that TPC and TFC were 141 µg eq. GA/mL, and 71 µg eq. QE/mL, respectively.

Key words: Papaya, Latex, Papain, Proteolytic activity, Antioxidant activity

INTRODUCTION

Papaya (Carica papaya L.) is believed to have originated in the tropical regions of South America and has since been extensively cultivated in tropical areas worldwide. This fruit is renowned for its diverse applications in medicine, nutrition, and healthcare. It contains a variety of bioactive compounds, including enzymes, organic acids, saponins, sugars, lipids, proteins, and carotenoids. Notably, papaya latex, leaves, fruits, and seeds have been utilized for their medicinal properties, such as antimicrobial, anti-inflammatory, and antioxidant activities (**Babalola et al., 2023).** Papain, a prominent cysteine protease enzyme found abundantly in papaya latex, is a globular protein composed of a single polypeptide chain with three disulfide bridges and a sulfhydryl group essential for its proteolytic activity. This enzyme exhibits remarkable stability under various conditions, including elevated temperatures. Papain has a wide range of therapeutic applications, including allergy treatment, wound debridement, and reduction of drug toxicity. Additionally, it is utilized in the food industry for meat tenderization and possesses notable antibacterial properties (**Babalola et al., 2024)**. In this study, papain was extracted and purified from papaya latex using Three-Phase Partitioning (TPP). Protein content and protease activity were assessed using Lowry's method and casein hydrolysis, respectively. The effects of pH, temperature, and enzyme concentration on protease activity, purification fold, and activity recovery were evaluated. Furthermore, the

proximate composition, phenolic content, and antioxidant activity of papaya latex were determined. Antioxidant activity was measured through reducing power, total antioxidant capacity, and DPPH radical scavenging assays.

MATERIALS AND METHODS:

A- Materials

Potassium-meta-bisulfite ($K_2S_2O_5$), ammonium sulfate, Folin reagent, bovine serum albumin, casein, Ascorbic acid (AA) and trichloroacetic acid (TCA) were purchased from Sigma-Aldrich without further modifications.

B- Methods

1- Papaya Latex (PL) extraction and collection

In the early morning, papaya latex (PL) was collected and extracted from unripe papaya fruit. The first cut depth ranged from 1 to 2mm, while PL flow stops after 4-6 min. The resulting PL was placed in a 250 mL beaker, and potassium-meta-bisulfite ($K_2S_2O_5$) was then added at a concentration of 0.5% to maintain papain stability. Five independent collections were made with different volumes: 12.3 mL, 10 mL, 9.4 mL, 9.5 mL, and 5.5 mL. The collected PL was dried for 2 hrs at 40 °C on petri dishes. The dried PL was then kept at -20°C until further analyses (**Rathi and Gadekar, 2007)**.

2- Isolation of papain

Papain isolation using three-phase partitioning was conducted by homogenizing PL (10 g) in 100 mL of 0.1 M phosphate buffer (pH= 7) using homogenizer (HG-15D-Set-A, Daihan, Taipei, Taiwan) followed by centrifugation at 5000 rpm for 30 min. The supernatant was then collected and the pellets were discarded. 10 mL of the t-butanol was added to 10 mL of the collected supernatant at 45º C. T-butanol was used to avoid denaturation of the partitioned enzyme as it is unable to permeate inside the folded three-dimensional structure of protein due to its larger molecular size (**Puig et al., 2008)**. Ammonium sulfate (8 g) was then added to the previous mixture under stirring at 200 rpm for 100 min, and the pH value was adjusted to 7. The solution was placed in a separating funnel and allowed to separate for 2 hrs at room temperature (± 25), and then three phases were observed. The top phase was discarded and the bottom and intermediate phases were collected. The ratio of 1:0.5 (V/V) was used to dissolve the intermediate phase in 0.1 M phosphate buffer with a pH of 7 (**Vetal and Rathod, 2015)**. Bottom phase was added to ethanol (96% v/v) in two proportions bottom phase: ethanol 1:3 to obtain a precipitate which was recovered by vaccum filtration using Wathman paper N°1. Finally, the solid was vacuum dried (at 50 °C, Lab live duo-vac oven, Lab Line Instruments), and the enzyme was ground to obtain a fine powder.

3- Measurement of protein content

The protein content was determined using Lowry assay (**Lowery, 1951)**. Aliquot 2.2 mL of fresh Lowry solution was added to 1.5 mL of the sample and then mixed well using a vortex. After 20 min of incubation at room temperature (± 25) in dark, 0.3 mL of the Folin reagent was applied to previous mixture. The solution was then incubated for 30 min in the dark at room temperature. After thoroughly blending the solution with a vortex, the absorbance was measured at 660 nm. Bovine serum albumin was used as a standard.

4- Evaluation and optimization of protease activity of extracted papain

The reaction mixture contained 200 µl of 50 mM cysteine, 20 mM EDTA (disodium salt) pH 7.0, 700 µl 50 mM Tris–HCl buffer (pH 7.0), and 100 µl enzyme solution. The mixture was incubated at 37 ◦C for 5 min before starting the reaction by adding 1 ml of 1% (w/v) casein solution. After 10 min, the reaction was stopped by adding 3 ml of 5% (v/v) trichloroacetic acid (TCA) and then cooled for 1 h. The reaction mixture was centrifuged and absorbance of the supernatant was measured at 275 nm.

Effect of pH on papain activity

To investigate the effect of pH on papain activity, buffer solutions were prepared at pH values of 6,0 ,6.5, 7,0, 7.5,8,0, 8.5 and 9.0 using appropriate buffer systems. For each pH condition, a reaction mixture was prepared by combining equal volumes of papain enzyme solution and substrate solution (1% casein), while maintaining the enzyme and substrate concentrations constant across all samples. Each reaction mixture was incubated at the optimal temperature for papain activity (60°C) for 10 minutes. After incubation, the enzymatic reaction was terminated by the addition of 10% trichloroacetic acid (TCA), which precipitated any undigested protein. The samples were then centrifuged, and the absorbance of the supernatant was measured at 280 nm using a spectrophotometer to quantify the amount of hydrolyzed protein, indicating enzyme activity **(McDonald and Chen, 1965).**

Effect of temperature on papain activity

To evaluate the effect of temperature on papain activity, a buffer solution was prepared and maintained at a constant pH of 6.5. Fixed

concentrations of papain enzyme and substrate (1% casein solution) were used for all samples to ensure consistency across temperature treatments. Aliquots of the reaction mixture were incubated at different temperatures: 30°C, 40°C, 50°C, 60°C, 70°C, 80°C and 90°C. Each reaction was allowed to proceed for 10 minutes. Following incubation, the enzymatic reaction was stopped by the addition of 10% trichloroacetic acid (TCA) to precipitate undigested protein. (**Murachi, 1970)**. The reaction mixtures were then centrifuged to remove precipitated proteins, and the absorbance of the supernatant was measured at 280 nm using a spectrophotometer.

Effect of Enzyme Concentration on Papain Activity

To assess the effect of enzyme concentration on papain activity, enzyme solutions were prepared at varying concentrations: 5 mg/mL, 20 mg/mL, and 40 mg/mL. The substrate concentration, pH (maintained at 6.5), and incubation temperature (60°C) were kept constant throughout the experiment. Each reaction mixture was incubated for 10 minutes, after which the reaction was terminated by the addition of 10% trichloroacetic acid (TCA). The samples were centrifuged, and the absorbance of the supernatant was measured at 280 nm to quantify enzyme activity (**McDonald and Chen, 1965).** In addition, proteolytic activities of the layers separated from the papaya latex were evaluated to investigate the most effective layer on the tested casein (**Vetal and Rathod, 2015)**. After determination of protein content and protease activity, activity recovery percentage and purification fold were calculated.

The activity recovery percentage quantifies the proportion of enzyme activity retained after a particular step (such as purification). It is calculated by comparing the enzyme activity after the step to the initial enzyme activity, then multiplying by 100 to express the result as a percentage.

$$\text{Activity Recovery}\,(\%) = \left(\frac{\text{Activity after treatment (units)}}{\text{Initial Activity (units)}}\right) \times 100$$

Activity after treatment (units): The enzyme activity measured after the purification step. Initial Activity (units): The enzyme activity measured before the start of the purification process. Units: This could be µmol/min, U/mL, or U/mg, depending on the method used to measure enzyme activity. The purification fold indicates how many times purer the enzyme has become after purification, by comparing the specific activity of the enzyme after purification to the specific activity before purification.

$$\text{Purification Fold} = \frac{\text{Specific Activity after purification (units/mg)}}{\text{Specific Activity before purification (units/mg)}}$$

Specific Activity after purification (units/mg): The enzyme activity per unit of protein after the purification step.

Units: This is usually expressed as U/mg (units of activity per milligram of protein).

Specific Activity before purification (units/mg): The enzyme activity per unit of protein before the purification step.

$$\text{Specific Activity} = \frac{\text{Enzyme Activity (units)}}{\text{Total Protein (mg)}}$$

5- Determination of antioxidant and antiradical activities of the PL

Determination of total phenolic content

Total phenolic content was determined using the Folin-Ciocalteu procedure according to **Singleton et al. (1999) and Stevanato et al. (2004).** The sample extract (0.1 mL), distilled water (7.9 mL), and Folin-Ciocalteu reagent (0.5 mL) were combined, and the mixture was neutralized with sodium carbonate (20%, 1.5 mL). Gallic acid was used as the standard for measuring absorbance at 765 nm after two hours in the dark.

Determination of total flavonoids

Colorimetric analysis was used to assess total flavonoid levels, according to **Chan et al. (2002)**. 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate, and 2.8 ml of distilled water were combined with the extract (0.1 ml). The mixture was kept at room temperature. The absorbance of the reaction mixture was measured at a wavelength of 415 nm.

The total antioxidant activity

According to the steps outlined by **Prieto et al. (1999)**. The phosphomolybdenum technique was used to assess the total antioxidant capacity. The assay is based on the reduction of Mo+6 to Mo+5 by the PL or ascorbic acid (AA), and subsequent formation of green phosphate/Mo (V) complex at acidic pH. Aliquot 0.1 mL of PL extract at different concentrations was combined with 1ml of reagent solution (0.6M H_2SO_4, 28 mM sodium phosphate, 4 mM ammonium molybdate mixture) in an Eppendorf tube. The tubes were then incubated for 90 min at 37 ºC. The mixture was then kept at room temperature, and the absorbance was measured at 695 nm in comparison with a blank. AA was employed as a reference.

DPPH radical-scavenging activity

The ability to scavenge 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) radical by PL extract was estimated to investigate the PL scavenging activity. A 0.1 mM DPPH solution was prepared in methanol and kept in the dark at room temperature (25 ± 1°C) until use. Papaya latex extract was dissolved in methanol With concentration (4mg/mL).1mL of DPPH reagent was mixed with 0.25 mL of 0.1 M Tris–HCl buffer (pH 7.4) and different aliquots of the sample were taken(50, 100, 150, 200 and 250 µL of sample (PL) in test tubes. The mixture was then gently mixed. The sample tubes were also incubated at room temperature in dark for 20 min. Ethanol was used as blank. The free radical-scavenging activity was calculated as decrease in the absorbance from the following equation (**Brand-Williams *et al.*, 1995).**

$$\text{Scavenging Activity (\%)} = \left(\frac{A_c - A_s}{A_c}\right) \times 100$$

A_c: Absorbance of the control (DPPH + methanol)
A_s: Absorbance of the sample (DPPH + papaya latex)

Reducing power assay

1 mL of PL or AA was combined with 2.5 mL of phosphate buffer (0.2 M, pH 7) and 2.5 mL of 1% potassium ferricyanide. The mixture was then incubated at 50ºC. Trichloroacetic acid (2.5 mL, 10%) was then added to the mixture and centrifuged for 10 min, followed by the addition of 2.5 mL of distilled water and 0.5 mL of a 0.1% ferric chloride solution. The reference standard used in this study was ascorbic acid. Absorbance was measured at 700 nm and compared to a blank solution (**Ferreira et al., 2007**).

Statistical Analysis

Statistical analysis was carried out using the Costat program version 6.311 (CoHort software, Monterey, USA) to find significant variations among the obtained results. In addition, statistical analysis of variance (ANOVA) was be helpful to compare all treatments. The standard deviation (SD) of the mean values was calculated, while Duncan's new range test at p = 0.05 was used to analyze the significant variations **(Duncan 1956; Gomez and Gomez 1984 and CoStat 2005).** In this test, every sample was analyzed by using three replicates for each one.

RESULTS AND DISCUSSION

Three phase partitioning (TPP) optimization

TPP was carried out to separate the papain enzyme into both bottom and intermediate layers with high purity, while the upper phase of t-butanol did not contain papain enzyme. Then, protease activities, protein contents, specific activities, activity recovery, and purification fold of the crude extract, bottom layer, and intermediate layer were determined to investigate the variation in these parameters based on the extract type. It was found that intermediate layer exhibited higher protease activity (5.99 $\times 10^{-2}$ U/mL) than other extracts. Whereas, bottom layer showed the lowest protease activity of 2.74 $\times 10^{-2}$ U/mL due to the variation in protein content, which showed higher concentration in both separated layers than that of crude latex, as listed in Table 1.

Similar results were observed by **Mahatara and Maskey (2023),** who mentioned that the intermediate layer protease activity was higher than that of bottom layer, which agree with the obtained results. However, the bottom layer obtained from the TPP of papaya peels showed higher protease activity than intermediate layer, which may be due to the variation in the plant part used in the study. In addition, intermediate layer showed higher activity recovery (153.58%) and purification fold (0.258) than the separated bottom layer.

Table 1. Enzymatic parameters of PL, and extracted bottom and intermediate layers.

Product	Protease activity (U/ml)	Protein content (mg/mL)	Specific activity (U/mg)	Activity recovery (%)	Purification fold
Crude extract	$3.9\times10^{-2}\pm0.003$ b	0.586	66.55×10^{-3}	-	-
Intermediate layer	$5.99\times10^{-2}\pm0.001$ a	3.78	17.2×10^{-3}	153.58	0.258
Bottom layer	$2.74\times10^{-2}\pm0.0009$ c	2.12	11.1×10^{-3}	70.25	0.166

Optimization of papain proteolytic activity

The effects of pH, temperature, and enzyme concentration on papain proteolytic activity were evaluated to determine the optimum parameters for papain catalytic activity (Table 2). In this respect, papain activity was tested at different pH values ranging from 6 to 9, and then the optimum pH value was used in further experiments to select the optimum temperature. Different temperatures in the range of 30-90 °C were used to study the effect of temperature on papain proteolytic activity. In addition, the optimum temperature was adjusted to evaluate the best concentration of papain enzyme for papain catalytic activity.

Table 2. Effect of pH value, temperature and papain concentration on proteolytic activity of papain

Experiment conditions			
pH value	Temperature (°C)	Papain Concentration (%)	Enzyme activity (U/ml)
6	70	2	$10.09\times10^{-2}\pm0.019^{b}$
6.5			$11.89\times10^{-2}\pm0.024^{a}$
7			$12.27\times10\pm0.013^{a}$
7.5			$11.49\times10^{-2}\pm0.009^{a}$
8			$9.1\times10^{-2}\pm0.009^{bc}$
8.5			$8.7\times10^{-2}\pm0.043^{c}$
9			$7.04\times10^{-2}\pm0.005^{e}$
7	30	2	$4.26\times10^{-2}\pm0.004^{g}$
	40		$5.48\times10^{-2}\pm0.003^{f}$
	50		$7.3\times10^{2}\pm0.006^{de}$
	60		$12.5\times10^{-2}\pm0.009^{a}$
	70		$12.27\times10^{-2}\pm0.013^{a}$
	80		$10.01\times10^{-2}\pm0.008^{bc}$
	90		$8.3\times10^{-2}\pm0.009^{cd}$
7	60	0.5	$7.8\times10^{-2}\pm0.005^{cde}$
		2	$12.27\times10^{-2}\pm0.013^{a}$
		4	$12.16\times10^{-2}\pm0.001^{a}$

Effect of pH

The effects of different pH values ranging from 6 to 9 on the proteolytic activity of papain enzyme were evaluated to determine the optimum pH value for the papain enzyme. In this respect, casein was used as the synthetic substrate. The highest proteolytic activity (12.16×10-2 U/ml) for papain enzyme was observed at pH 7, as shown in Table 2, which means that the neutral solution was the most appropriate medium for papain activity. There were no significant differences between the proteolytic activities of papain at pH 6.5, 7, and 7.5. However, the proteolytic activity of papain significantly decreased as the pH increased above 7.5. Similar results were observed by **Khatun et al. (2023)**. Based on the results listed in Table 2, the following investigations to optimize the parameters of papain proteolytic activity were carried out at pH 7, as the optimum pH value, as shown in Fig. 1.

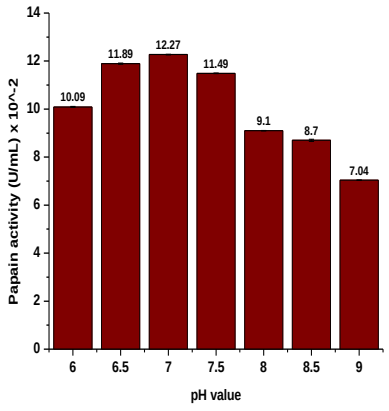

Fig. 1. The effect of different pH values on the proteolytic activity of papain

Effect of temperature

Temperature is an important parameter that affects the enzyme configuration and overall stability (**Khatun et al., 2023)**. The effect of temperature on the proteolytic activity of papain was studied to determine the optimum temperature for papain activity. Different temperatures ranged from 30 °C to 90 °C, were used to investigate the optimum temperature. From the results obtained from this study and listed in Table 2, it was found that the optimum temperature was 60 °C with the highest proteolytic activity value of 12.5×10-2 U/ml. The reduction in temperature lower than 60 °C led to significant decrease in the proteolytic activity of the papain enzyme. The decrease in proteolytic activity with the decrease in temperature may be due to low temperature not enough for enzyme to function **(Maria et al., 1997)**. While, there was no significant variations between the proteolytic activities at 60 and 70 °C, indicating that the best range of temperatures was between these temperatures, as shown in Table 2. Similar results were mentioned by **Mahatara and Maskey (2023),** who reported that there was no difference between the proteolytic activity at 60 °C and 70°C, which resulted in the highest activity, as displayed in Fig. 2. In addition, an increase in the temperature above 70 °C significantly minimized the proteolytic activity of papain. The reduction in proteolytic activity due to the increase in temperature may be due to high temperature the rate decreases again because the enzyme becomes denatured and can no longer function. **(Maria et al., 1997)**.

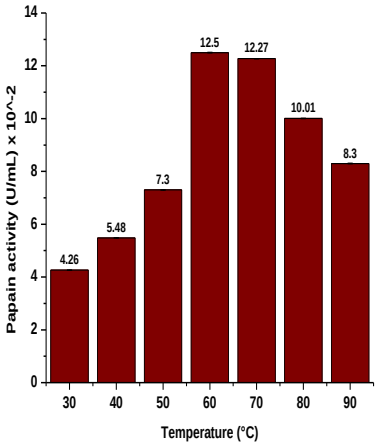

Fig. 2. The effect of different temperatures on the proteolytic activity of papain.

The optimum temperature of 60 °C was selected for the next study to evaluate the papain concentration effect on the proteolytic activity of papain, as displayed in Fig. 2.

Effect of papain concentration

Casein is commonly used to determine the nonspecific proteolytic activity of enzymes. Furthermore, caseinolytic activity was determined to evaluate papain activity. Table 2 shows the effects of different enzyme concentrations on papain proteolytic activity. In general, there was an improvement in caseinolytic activity with an increase in papain concentration. However, no significant difference ($p < 0.05$) was observed between the assays with 2% and 4% papain concentrations, as shown in Fig. 3. Moreover, the increase in proteolytic activity was not proportional to an increase in papain concentration. The present results are similar to those of other studies, such as those of **Foda (2016).** In the last study, for example, the authors demonstrated that an increase in papain concentration improved the proteolytic activity of papain up to 2 % (v/v), as illustrated in Fig. 3.

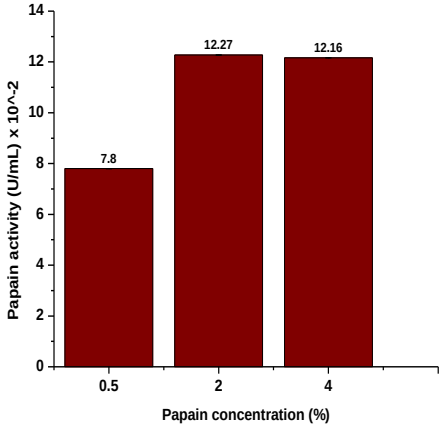

Fig. 3. The effect of different papain concentrations on its proteolytic activity.

Evaluation of antiradical and antioxidant activities of PL Total phenolic content

The Folin–Ciocalteu method is typically used to detect phenolic compounds, which are thought to be the main class of naturally occurring antioxidants found in plants. Total phenolic content was analyzed and expressed as microgram equivalent gallic acid per mL of PL (µg eq. GA/mL). The total phenolic content of PL was 141 µg, as shown in Eq. GA/ml, respectively, as shown in Table 3. The amount of phenolic compounds in plants is a crucial factor as an antioxidant component because of their significant ability to scavenge free radicals, which confirms the potential of PL as an antioxidant (**Ruining et al., 2022).**

Total flavonoid content

Flavonoids comprise the majority of polyphenols. It is commonly recognized that the majority of plants have radical scavenging properties due to the presence of flavonoids. Total flavonoids content in PL was determined and expressed as microgram equivalent quercetin per mL of PL (µg eq. QE/mL). Table 3 showed that the total flavonoids content was 71 µg eq. QE/ml. The presence of flavonoids increases the abundance of hydroxyl groups and their replacement with electron-donating alkyl or methoxy groups, which enhances the antioxidant activity of PL (**Ruining et al., 2022).**

Table 3. Total phenolic and total flavonoid contents of PL

Total phenolic content (µg eq. GA/ml)	Total flavonoid content (µg eq. QE/ml)
141	71

Total antioxidant capacity

Phosphomolybdenum assay is a spectrophotometric method used to evaluate the antioxidant activity of PL. This assay is based on the reduction of Mo^{+6} to Mo^{+5}, using antioxidant compounds found in the PL **(Foda, 2016)**. In this study, the antioxidant capacity of PL was compared to ascorbic acid (AA) as a control at different concentrations ranged from 5 to 25 µg/mL. The increase in absorbance is considered as indicator for the increase of total antioxidant activity of the tested sample. The results showed that increasing the concentration of the PL increased significantly its antioxidant capacity. In general, the antioxidant capacity of PL was lower than that of AA. Average values of 0.268 and 0.485 were detected for the absorbance of PL and AA, respectively, at a concentration of 25 µg/mL, as listed in Table 4. However, PL showed higher antioxidant activity than AA at a low concentration of 5 µg/mL.

Table 4. Total antioxidant capacity comparing PL with ascorbic acid.

Concentration (µg/mL)	Absorbance	
	PL	AA
5	0.032 ±0.003 e	0.004 ±0.001 e
10	0.091 ±0.002 d	0.124 ±0.003 d
15	0.150 ±0.005 c	0.244 ±0.002 c
20	0.209 ±0.003 b	0.365 ±0.001 b
25	0.268 ±0.11 a	0.486 ±0.003 a

DPPH scavenging capacity

The ability of a tested compound to scavenge free radicals was determined by its ability to scavenge. Compared to other

approaches, this approach is frequently employed to assess antiradical activity in a comparatively short amount of time. When DPPH, a stable free radical, comes into contact with antioxidants, it transforms into 1, 1-diphenyl-2-(2, 4, 6-trinitrophenyl) hydrazine. The discoloration reveals an antioxidant compound's capacity for scavenging (**Phanat et al., 2012).** The results showed that the PL exhibited a free radical scavenging capacity ,The IC_{50} value of the PL was 223.75 µg/mL, as tabulated in Table 5. The results of this study suggest that PL may have potential as a natural antiradical. This means that the PL is able to scavenge free radicals at different concentration.

Table 5. DPPH scavenging ability in PL.

Concentration (µg/ml)	Inhibition (%)	IC_{50} (µg/ml)
25	10.27 ±0.02 f	223.75
50	13.24 ±0.01 e	
100	25.59 ±0.023 d	
150	31.26 ±0.02 c	
200	43.25 ±0.023 b	
250	55.29 ±0.02 a	

Reducing power assay

The reducing power assay is a simple and rapid method used to measure the antioxidant activity of a sample. It is based on the principle that antioxidants can donate electrons to free radicals, thereby preventing them from damaging cells. The assay measures the ability of a sample to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions (**Phanat et al., 2012).** The increase in the mean of the absorbance value of the tested sample is an indicator for the maximization of reducing power of the tested sample. The reducing power capacity of PL and AA augmented significantly as the increase in their concentration from 10 to 75 µg/ml, as tabulated in Table 6. At all tested concentrations the reducing power of PL was lower than that of AA, which was used as a reference and highly potential antioxidant.

Table 6. Reducing power in PL

Concentration (µg/ml)	Absorbance	
	LE	AA
10	0.113 ±0.004 d	0.161 ±0.004 d
25	0.571 ±0.002 c	0.783 ±0.008 c
50	0.696 ±0.003 b	1.647 ±0.005 b
75	0.771 ±0.003 a	2.212 ±0.005 a

CONCLUSION

Proteolytic enzymes contained in papaya fruit latex can be simply extracted using phosphate buffer pH 7 from the crude latex. Intermediate layer showed the highest proteolytic activity, while crude latex exhibited greater specific activity than that of bottom and intermediate layers due to the variation in protein content, which showed higher concentration in both separated layers than that of crude latex. In addition, intermediate layer showed higher activity recovery and purification fold than the separated bottom layer. Additionally, pH 7, 60ºC and a papain concentration of 2% were the optimum parameters for protease activity of the papain enzyme. The antioxidant activity, DPPH scavenging activity, and reducing power of PL increased significantly with increasing PL concentration. Furthermore, the TPC and TFC of PL were found to be 141 µg eq. GA/mL, and 71 µg eq. QE/mL, respectively.

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CONFLICTS OF INTEREST:

The authors declare that they have no conflict of interest.

AUTHORS CONTRIBUTION

All authors developed the concept of the manuscript, shared writing. All authors checked and confirmed the final revised manuscript.

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تعظيم الاستفادة من لاتكس البابايا ونشاط التحلل البروتيني للغراء وتقييم أنشطة مضادات الأكسدة في اللاتكس

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أحد الإنزيمات الرئيسية الموجودة في البابايا هو البابايين، والذي يستخدم في العديد من المجالات المختلفة. لذلك، من أجل استخدام الغراء يجب جمعه وعزله وتنقيته من نباتات البابايا. تتناول هذه الدراسة استخلاص وتنقية الغراء من لاتكس البابايا .(PL) تم استخدام تقنية التقسيم ثلاثي الطور (TPP) في الاستخلاص والتنقية. تم تحديد نشاط الأنزيم البروتيني، ومحتوى البروتين، والنشاط المحدد، واستعادة النشاط، وأضعاف التنقية لكل من الطبقتين المتوسطة والسفلية لدراسة التباين في هذه العوامل في الطبقات المنفصلة من اللاتكس. ثم، بعد استخراج إنزيم غراء من اللاتكس، تم اختبار آثار المعلمات المختلفة، بما في ذلك درجة الحموضة ودرجة الحرارة وتركيز غراء، على نشاط الأنزيم البروتيني لتحسين نشاط غراء. وفي هذا الصدد، وجد أن الرقم الهيدروجيني 7، 60 درجة مئوية وتركيز 2٪ كانت المعلمات المثلى لنشاط الأنزيم البروتيني لإنزيم البابايين. بالإضافة إلى ذلك، تم تقييم نشاط مضادات الأكسدة للـ PL المستخرج من ثمار البابايا باستخدام إجمالي نشاط مضادات الأكسدة، وتقليل الطاقة، ومقايسات .DPPH زاد نشاط مضادات الأكسدة وتقليل الطاقة ونشاط تنظيف DPPH لـ PL تدريجيًا مع زيادة تركيز .PL علاوة على ذلك، تم تحديد إجمالي محتوى الفينول (TPC) ومحتوى الفلافونويد الإجمالي (TFC) لـ PL لتأكيد نشاط مضادات الأكسدة المحتملة لـ .PL وقد وجد أن TPC و TFCكانا 141 ميكروغرام مكافئ/GA .مل، و 71 ميكروغرام مكافئ. التيسير الكمي/مل، على التوالي .