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Study on Measuring Proteolytic Activity and Phyto Chemical Analysis of Fruit Latex and Leaves Extract of Papaya (*Carica papaya* L.*)* Cv. CO_2

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ABSTRACT

Background: Papaya latex is a milky fluid secreted by ducts and is a complex mixture of phytochemicals (secondary metabolites). Lowry's method was used to quantify the papain extracts from the fruits and leaves to measure cysteine protease activity.

Methods: The experiment was conducted using the extracts of papaya fruit latex (Papain) and leaf extracts for phytochemical analysis. As a result, the enzyme concentration in crude extracts was highest in the fruit latex and lowest in the leaves. The dialyzed leaves extract band/color was very light in comparison to the fruit latex band/color, indicating that the enzyme concentration in the crude leaves sample was the lowest.

Result: Quantitative estimation of protein content was observed higher in papaya fruit latex papain (82.5 mg/ml) and in papaya leaves (36.6 mg/ml). Positively higher significant results were also confirmed for the protease activity examined for latex and leaves valued 6.34 mg and 5.323 mg, respectively, whereas, the similar results were noted for the content of phytochemical constituents in fruit latex for glycosides, saponins and terpenoids as well as in leave extract for glycosides, flavonoid, phenol, reducing sugar, tannins, terpenoids, saponins.

Key words: Extract, Latex, Papain, Phyto-chemical, Proteins, Proteolytic, Quantitative estimation, Sdg7.

INTRODUCTION

Papaya (*Carica papaya* L) belongs to the flowering plant taxa classified in the order brassicales and remains in family Caricaceae with 6 genera and 35 species by using the 'Flora of Marathwada,' their identification was confirmed by Naik, (1998). Papain production is typically derived from the latex of unripe papaya fruits but other sources such as fruit peels, leaves, petioles, stems and bark are still being studied for papain activity (Baeza *et al.,* 1989). The analysis of other parts of the papaya plant has become important due to the laborious and time-consuming process of extracting latex from the fruit (Chaiwut *et al.,* 2007). The presence of proteolytic activity in papaya peel extracts has been discovered, as well as the possibility of producing crude papain from pawpaw peels (Chaiwut *et al*., 2007). A correlation study revealed that among the various varieties, the most promising yield was offered and seen in long-fruited ones, with fruit length having a greater influence than circumference (Irulapppan, 1980). Various biological activities in fruits, their role in cystenine proteases and protection (Almehmadi, 2023), these aspects affects simultaneously in papain yield for fruit maturity. Papain yield was maximum from fully grown yet unripe papaya fruits, as revealed by Espin and Islam (1998). Papain's arousing characteristics allow it to lead peptides, larger amounts of identified source, thiol enzyme and fungal source cysteine enzymes over longer periods of time (Market Research Future 2017 and Global Meat Tenderizing Agent Market Research Report-Forest 2023). Latex is produced by around 10% of flowering plants and is present in over 40 families, including the Euphorbiaceae, Apocynaceae, Caricaceae, Moraceae and Asclepidaceae (Agrawal and Konno, 2009). The latex is a milky fluid secreted by ducts of laticiferous tissue and flowing inside laticifers such as leaves, stems, fruits and roots of various blooming plants (Hagal *et al.,* 2008) and Pickare, (2008). Latex is a complex mixture of secondary metabolites that contains a variety of physiologically active chemicals and antibacterial properties (Santos *et al*., 2022). Secondary plant metabolites (phytochemicals) have been intensively studied as a source of therapeutic medicines in recent years (Balandrin *et al.,* 1985; Kanokwiroon *et al*., 2008 and Siritaperawee *et al*., 2012). Proteins, alkaloids, tannins, terpenes, starch, sugars, oils, resins, gums and enzymes are some of the known constituents of latex (Pandey, 2001). Because tribal

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communities use plant latex, it has a broader ethnopharmacological applicability as a result, the goal of this research is to identify latex phytochemical components (Om *et al.,* 2008).

MATERIALS AND METHODS

An experiment in laboratory was carried out on research entitled "Study on Measuring Proteolytic Activity and Phyto Chemical Analysis of Fruit latex and Leaves Extract of Papaya (*Carica papaya* L.*)* cv. CO_2" in department of Horticulture, Lovely Professional University, Phagwara, Jalandhar, Punjab during the year 2021-2022. The use of preservatives improves the activity and quality of sun-dried crude papain. Colour/appearance, fragrance and enzymatic activity all are improved by the preservatives. Sodium meta-bisulfide when used @ 0.1 per cent (w/v) produces good results.

Qualitative phytochemical analysis

Latex is a complex mixture of secondary metabolites (Santos *et al.,* 2022) that contains a variety of physiologically active chemicals and antibacterial properties. Qualitative chemical analyses were conducted. (Kanokwiroon *et al*., 2008 and Siritaperawee *et al.,* 2012). Secondary plant metabolites (phytochemicals) have received a lot of attention in recent years as a potential source of therapeutic medicines (Balandrin *et al*., 1985). Proteins, alkaloids, tannins, terpenes, starch, sugars, oils, resins, gums and enzymes are some of the known constituents of latex (Pandey, 2001). Plant latex has a broader ethnopharmacological applicability since tribal people use it to identify different phytoconstituents using standard procedures (Evans, 1996; Kokate *et al.,* 1999 and Siddiqui and Ali, 1997).

Collection of latex

The samples are first extracted and dried in Mahadevpur, Telangana, using cultivar CO_2, Early in the morning, latex samples were taken from each plant species by nipping the leaves or making incisions 2 mm in the fruit latex extracted and dried papain of 75 and 105 days after fruit set as shown in Plate 1, using stainless steel razor blades and collected in plastic boxes and latter letting the latex drain in a sterile glass tube separately. The samples were delivered to the lab and maintained in the refrigerator at 4°C until they were used.

To improve appearance, colour and operation, crude latex was mixed with following chemicals,0.1 per cent sodium metabisulphite ($Na_2S_2O_5$) and 0.1 per cent benzoic acid (Kabuo *et al*., 2003). 40 mM Cystein, 6 M Hydrochloric acid (HCl), 6 M sodium hydroxide (NaOH), Ammonium sulfate (NH4) $2SO_4$, 45% saturation, sodium chloride(Nacl) 10%, 20 mM Cystein, Folin-Ciocalteau Reagent (FC) 0.5 mM (0.3 ml), 0.2 M tris buffer100 Mm PBS,fresh lowry solution (2.2 ml), normal standard bovine serum albumin (BSA) 10 mg/ml, Trichloroacetic acid (TCA) 5 mL 5%, sodium carbonate 5 mL, Tyrosine (10 mg/ml) Hydroalcohol solution 1l0.65 per cent casein, 500 papain extract L, Trichloroacetic acid (TCA) 5 mL5%, sodium carbonate 5 mL.For photochemical analysis, latex was homogenised in a homogenizer and filtered through four folds of muslin cloth. The addition of potassium metabisulphite (0.05%) and benzoic acid (0.1%) to liquid latex improves the appearance and colour of sun-dried papain and extends its storage life of papain. When a pawpaw fruit was first tapped, one incision was made and consecutive extractions increased the number of incisions. Latex was frozen at -20°C to -8°C in the lab to prevent enzyme degradation until it was ready to be used for chemical analysis (Narinesingh and Maraj, 1989).

Grinding and Ultra-sonication method

Fresh papaya leaves were sliced and washed in a distilled water bath. Then it was dried for seven days at room temperature using atmospheric air. A grinder was being used by the leaves. 5 g of ground papaya leaf powder was accurately dissolved in 20 ml distilled water, the water was added in a 1:5 ratio and the liquid was filtered using filter paper at 50°C for 50 minutes. Later the samples were grounded and pre-treated with ultrasonication for 60 minutes at an extraction temperature of 60°C).

Plate 1: Cultivar CO2 used for extraction of papain from fruits (ml).

Dialysis

The solution was placed in a dialysis bag and the sample inside was checked for leakage. The dialysis bag was then suspended in a beaker filled with a 100 mM phosphate buffer-NaCl solution. This arrangement was kept chilled in the refrigerator overnight. The whole technique was followed for both leaves and fruit latex papain. 1000 ml of 100 mM PBS is introduced. The concentration of Tris buffer within the cassette falls below 0.01 M as the PBS buffer diffuses in and the Tris buffer diffuses out. Finally, in a freeze dryer, the six dialyzed extracts were frozen overnight at 40°C. We now have papain powder that has been refined. Each papain extract's specific activity was determined by dividing the proteolytic activity by the protein concentration in the same phase. The purification fold was determined by dividing the phase extract's specific activity by the crude sample's specific activity.

RESULTS AND DISCUSSION

This study was focused on investigating phytochemical properties of latex and Phytochemical analysis of latex is represented below.

Quantitative estimation of enzymes in the crude and dried samples by Lowry's method

The enzyme content in the crude extracts and dried samples of the fruit and leaves was determined to be 82.5 mg/ml and 36.6 mg/ml, respectively, using Lowry's method to quantify the papain extracts from the fruits and leaves. As a result, the enzyme concentration in crude extracts was highest in the fruit latex and lowest in the leaves as indicated in Table 1. The enzyme assay was then used to measure the activity of the crude extracts, which revealed that the leaves had the highest activity. By using ammonium sulphate precipitation and dialysis, the crude extracts and dried papain enzyme samples were purified. The dialyzed leaves extract band/colour was very light in comparison to the fruit latex band/colour, indicating that the enzyme concentration in the crude leaves sample was the lowest, which was consistent with Lowry's technique result as shown in Plate 2. And they learnt how to purify papain from the fruits and leaves using a two-step salt precipitation method. The pure latex content was highest in the fruit latex and lowest in the leaves, as can be shown. Because the crude enzyme contains other proteins, the purified papain concentration was lower than the crude enzyme concentration as shown in Table 1. The

Plate 2: Colour band of fruit latex and leaf extract for protein estimation.

Table 1: Absorbance of standard bsa to determine the concentrations of latex from fruits and leaves of papaya.

Volume of Std BSA (mg/ml)	Volume of distilled water (mg/ml)	OD at 660 nm
0	10	0
0.5	9.5	0.07
1.0	9	0.15
1.5	8.5	0.2
2.0	8	0.29
2.5	7.5	0.33
Test samples(mg/ml)		
10 (Latex)	8.5	1.08
10 (Leaves)	8.5	1.29

increase in temperature over the optimal temperature may have caused the peptide bonds to break down, resulting in the enzyme's inactivity. As a result, the optimum temperature for papain activity was 65°C. In comparison to our results, the latex of the papaya contains a higher concentration of enzyme than the leaf as presented in Sarote *et al.,* (2006) revealed that extracting papain enzyme from the leaf is easier than extracting papain enzyme from the latex. Previously, experiments were carried out for a set amount of time, but the effect of reaction time between the enzyme and the substrate has also been explored. After 20-30 minutes of incubation with substrate, the activity of crude extract and dried samples of papain was investigated. The results revealed that activity increases after 30 minutes of incubation and then settles into a steady level, as seen in Plate 4. The product generated by enzyme activity on substrate is similarly time-dependent, increasing with time until it achieves the reaction's minimum time requirement. As a result of the findings, 30 minutes may be the ideal duration for enzyme activation.

Determination of enzyme activity of crude papain and dried samples of latex and leaves of papaya

Spectrophotometer at 660 nm was used to determine papain's proteolytic activity. The goal of the proteolytic activity is to see how well papain can break down molecules. UV light can be consumed by either an object or a reactant at the point where it is given in the example. If the reactant absorbs the light, the absorbance decreases. As a result of Tyrosine's assimilation of UV radiation, the absorbance value has increased. Proteolytic action is achieved by combining papain with cysteine. After adding cysteine, the time it takes to get the product was lowered, according to the results.

The amount of papain enzyme extracted from grinded papaya leaves was higher than in sonicated samples, which could be due to the grinding of the leaf avoiding the outer regions of the leaf that contained the cytoplasm. In comparison to the long processing period, highly pure papain was obtained in a shorter period (Sarote *et al.,* 2006) The papain enzyme activity is higher in papaya leaf samples than in papaya latex as shown in Table 2. This is consistent with the current investigation, which found that the sonicated leaf samples had higher papain enzyme efficiency than the grinded leaf samples, which could be due to papain enzyme contamination with the grinded particles. As the temperature and length of sonication increased, so did the concentration of papain enzyme as indicated in this revealed that the breakdown of cellular components was aided by warmth (Sarote *et al.,* 2006). In comparison to the results of the preceding methods, the latex of papaya contains a larger quantity of enzymes than the leaf (Sarote *et al.,* 2006). Papain enzyme extraction from the leaf is easier than papain enzyme extraction from the latex component. Protease activity rises in tandem with temperature rises until the optimum temperature is reached, at which point further temperature rises to reduce protease activity. The enzyme activity is poor at lower temperatures than the optimal temperature, because there is less activation of kinetic energy available, however, in this case the kinetic energy required to maintain active complex conditions, includes both

Table 2: Determination of enzyme activity of crude papain and dried samples of latex and leaves of papaya.

Volume of Std tyrosine (µg/ml)	Volume of distilled water (µg/ml)	OD at 660 nm	Activity in its (per mg)
0	1	0	0
0.2	0.8	0.06	1.2285
0.4	0.6	0.17	1.7376
0.6	0.4	0.25	1.7059
0.8	0.2	0.34	1.7195
Test samples (mg/ml)			
10 (Latex)	1	1.04	6.3463
10 (Leaves)	1	1.24	5.3236

Plate 3: Dialysis membrane with the papain samples of extracted latex from unripe fruits and leaves of papaya.

enzyme and substrate molecules. Temperature impacts the pace of enzyme catalysis reactions in two ways (Hames and Hooper, 2000). Temperature increases the thermal energy of substrate molecules, which speeds up enzyme processes. Increased temperature also affects the structural changes of the substrate, making it more difficult for the substrate to access the enzyme's active site, resulting in a decrease in enzyme activity.

Purification of freshly extracted latex of fruit/leaf samples and determination of purity

dialysis

For purification of papain from clarified latex, a version of a two-step salt precipitation process provided (Baines and Brocklehurst, 1979) was adopted. Ammonium sulphate was utilised for precipitation, while sodium chloride was employed for the second stage, which resulted in an increase in protease activity (including papain). Papain of poorer purity was recovered in the precipitate. At 45 per cent saturation with ammonium sulphate, a maximum of approximately half the protease activity found in the latex was precipitated. Finally, dialysis was performed and lyophilized in a deep freezer at 40°C overnight, revealing the crude extracts' results. Dried papain enzyme samples were purified by ammonium sulphate precipitation and dialysis. The dialyzed leaves extract band/color was very light in comparison to the fruit latex band/color, indicating that the enzyme concentration in the crude leaves sample was the lowest, which was consistent with the Lowry's technique result, which demonstrates papain's purity (Plate 3).

Phyto chemical analysis of extracts of fruit latex and papaya leaves

Carica papaya, among other herbal plants, is well renowned for its therapeutic benefits in traditional medicine. The goal of this study was to look into the phytochemical features of latex (Plate 4). Crude latex and dried papain powder of papaya showed phytochemical analysis of latex as presented in Table 3. Saponin, terpenoid and glycosides were found in papaya (Sibi *et al*., 2013). Saponin, flavonoids, reducing sugar, tannins, phenols, terpenoids and glycosides have all been detected in leaf extracts. The leaf extract had the highest presence of chemicals in the phytochemical tests conducted (Pedro, 2011).

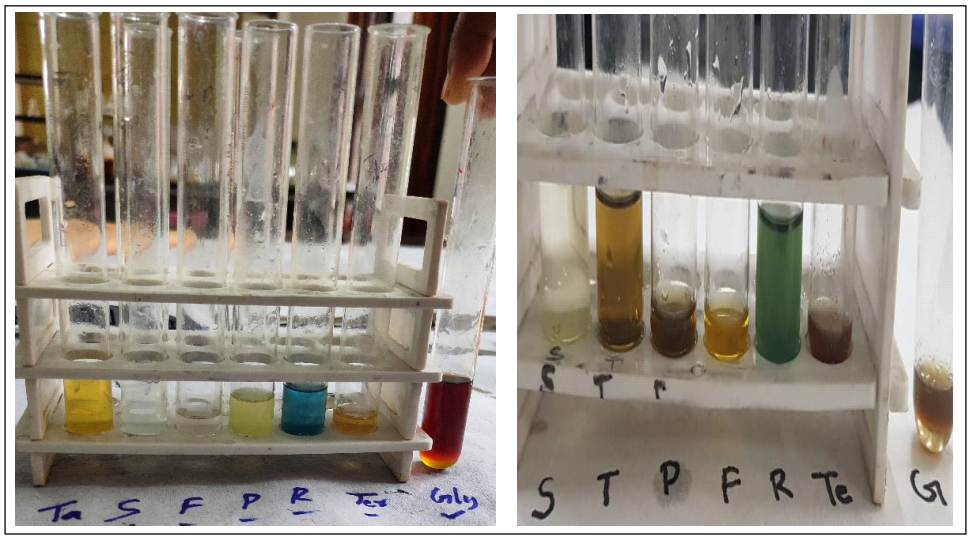

Plate 4: phytochemical constituents of fruit latex and leaf extracts of papaya.

Table 3: Phyto chemical constituents of fruit latex and leaf extracts of papaya.

Phytochemicals	Inferences
Flavonoids	Colour change from pale red to crimson
Glycosides	Presence of brick red precipitate
Phenols	Colour changes to blue-green/black
Reducing sugars (Benedict's test)	Presence of green colour
Saponins	Frothing extract which persisted for 15 minutes
Tannins	Colour changes to black green
Terpenoids	Formation of brick red precipitate to greyish colour

CONCLUSION

The goal of this research was to extract, dry and purify papain enzyme from papaya fruits and leaves, as well as exploit out the best ways to use the enzyme's activity and concentration. Proteolytic enzymes found in papaya fruit latex may be easily eliminated since crude papain's activity was higher than papain purified by two-stage salt precipitation and extraction and purification of papain under ideal circumstances. From current experiment, it can be concluded that enzyme activity assays for crude extract and dried samples of Papain, as well as their effect on enzyme activity proves to be the best which in turn gives the desired results and reveals that papain enzyme from fruit has the highest activity (6.3463/mg), hence proves that purified latex papain has stronger proteolytic activity than leaves.

Conflict of interest: None.

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