International Journal for Modern Trends in Science and Technology
Volume 12, Issue 02, pages 89-93.
ISSN: 2455-3778 online
Available online at: http://www.ijmtst.com/vol12issue02.html
DOI: https://doi.org/10.5281/zenodo.21589356

Comparative Assessment of Protease, Antibacterial and Antioxidant Activities of Pineapple *(Ananas comosus)*, Papaya *(Carica papaya)* and Kiwi *(Actinidia deliciosa)* Fruit Extracts

Shaik Parveen Banu | J. Ajani

Department of Biochemistry, Yogi Vemana University, Vemanapuram, Kadapa – 516005, Andhra Pradesh, India.
parveenbio1982@gmail.com , ajanireshma@gmail.com

To Cite this Article
Shaik Parveen Banu & J. Ajani (2026). Comparative Assessment of Protease, Antibacterial and Antioxidant Activities of Pineapple *(Ananas comosus)*, Papaya *(Carica papaya)* and Kiwi *(Actinidia deliciosa)* Fruit Extracts. International Journal for Modern Trends in Science and Technology, 12(02), 89-93. https://doi.org/10.5281/zenodo.21589356

Article Info
Received: 23 January 2026; Revised: 21 February 2026; Accepted: 26 February 2026.

KEYWORDS

Bromelain; Papain; Actinidin; Folin–Ciocalteu method; Disc diffusion; DPPH radical scavenging activity

ABSTRACT

Fruits form an integral part of the human diet and are widely recognised for their nutritional and pharmacological value. The present study was undertaken to compare the protease, antibacterial and antioxidant activities of three commonly consumed fruits — pineapple (Ananas comosus), papaya (Carica papaya) and kiwi (Actinidia deliciosa). Protease activity of bromelain, papain and actinidin extracted from the respective fruits was estimated by the Folin–Ciocalteu method using sodium caseinate as substrate and tyrosine as the reference standard. Antibacterial activity of the fruit extracts was evaluated against the Gram-positive bacterium Staphylococcus aureus and the Gram-negative bacteria Escherichia coli and Klebsiella sp. by the Kirby–Bauer disc diffusion method. Antioxidant activity was assessed by the DPPH free-radical scavenging assay, with ascorbic acid as the positive control. Papain from papaya exhibited the highest protease activity (99.343 µmol/mL/min), followed by bromelain from pineapple (55.190 µmol/mL/min) and actinidin from kiwi (5.519 µmol/mL/min). Pineapple extract produced the largest zones of inhibition against all three test organisms, with the greatest inhibition recorded against S. aureus (19 mm), followed by E. coli (18 mm) and Klebsiella sp. (16–17 mm); all three extracts inhibited the Gram-positive organism more effectively than the Gram-negative organisms. Pineapple extract also showed the highest DPPH radical scavenging activity (83.02%), followed by papaya (81.77%) and kiwi (60.46%). These findings indicate that pineapple, papaya and

I. INTRODUCTION

Regular consumption of fruits and fruit juices is widely recommended as part of a healthy diet, owing to their contribution to digestion, iron absorption and appetite stimulation. Beyond their nutritional role, several fruits are valued for bioactive compounds with pharmacological relevance, including proteolytic enzymes, antimicrobial phytochemicals and antioxidants that help mitigate oxidative stress associated with a range of chronic diseases. These properties have supported the growing use of fruit-derived compounds in the pharmaceutical, food-processing and cosmeceutical industries.

Pineapple (Ananas comosus), papaya (Carica papaya) and kiwi (Actinidia deliciosa) are tropical and sub-tropical fruits that are cultivated widely and consumed globally. Each of these fruits contains a distinct cysteine protease — bromelain in pineapple, papain in papaya and actinidin in kiwi — that has found application in meat tenderisation, protein hydrolysis, wound debridement and digestive-aid formulations [1–8]. Recent work continues to refine the comparative kinetic and functional characterisation of these enzymes; for instance, actinidin has been shown to hydrolyse whey and milk proteins with kinetic and thermodynamic behaviour distinct from that of bromelain and papain, supporting its potential in dairy protein processing [18].

In addition to their enzymatic activity, extracts of these fruits have demonstrated antibacterial action against pathogenic Gram-positive and Gram-negative bacteria, and antioxidant activity attributable to their phenolic, flavonoid and ascorbic acid content [9–17]. Fermented and co-enzyme preparations derived from pineapple and papaya waste have also been reported to retain notable antibacterial and antioxidant potential, highlighting the value of these fruits even beyond their edible pulp [19]. Continued interest in plant-derived antimicrobial agents as adjuncts or alternatives to conventional antibiotics has been reinforced by recent reports on plant-derived compounds and essential oils acting against clinically relevant Gram-negative organisms such as Escherichia coli [22, 23].

Despite the individual documentation of these properties, comparative studies evaluating protease, antibacterial and antioxidant activities of pineapple, papaya and kiwi extracts side by side, under identical experimental conditions, remain limited. The present study was therefore designed to assay and compare the protease activity (bromelain, papain and actinidin), antibacterial activity (against S. aureus, E. coli and Klebsiella sp.) and antioxidant activity (DPPH radical scavenging) of pineapple, papaya and kiwi fruit extracts, with a view to identifying their relative pharmacological potential.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh, ripe fruits of pineapple (Ananas comosus), papaya (Carica papaya) and kiwi (Actinidia deliciosa), of uniform size and free of visible defects, were procured from a local retail market. The fruits were transported to the laboratory and processed on the day of collection.

2.2 Chemicals and Reagents

Phosphate buffer (0.1 M, pH 7.0), phosphate-buffered saline (0.1 M, pH 7.0), sodium caseinate, trichloroacetic acid, sodium hydroxide, Folin–Ciocalteu reagent, L-tyrosine, nutrient broth, nutrient agar, ascorbic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were of analytical grade. Standard bacterial cultures of Staphylococcus aureus, Escherichia coli and Klebsiella sp. were used as test organisms.

2.3 Preparation of Fruit Extracts

Fruits were washed and manually peeled, and the pulp was separated and stored at 4 °C. Fifty grams of pulp from each fruit was homogenised in an electric blender and filtered through muslin cloth. Twenty millilitres of the resulting homogenate was centrifuged at 13,000 rpm for 15 min at 4 °C, and the supernatant was made up to 50 mL with the appropriate phosphate or PBS buffer. The resulting extracts were used for protease, antibacterial and antioxidant assays.

2.4 Assay of Protease Activity

Protease activity of the extracts (bromelain from pineapple, papain from papaya and actinidin from kiwi) was estimated by a modified Folin–Ciocalteu method. An aliquot (100 µL) of each buffered extract was

incubated with 1 mL of 1% (w/v) sodium caseinate at 37 °C for 10 min in the presence of 6 mM EDTA, and the reaction was terminated with 5% trichloroacetic acid. Following centrifugation, the supernatant was treated with 0.5 N NaOH and Folin–Ciocalteu reagent, and the resultant blue colour was read spectrophotometrically at 700 nm. Tyrosine (200–1000 µg) served as the reference standard, and protease activity was expressed as µmol/mL/min. All assays were performed in triplicate and the reported values represent the mean of three independent determinations.

2.5 Determination of Antibacterial Activity

Antibacterial activity was assessed by the Kirby–Bauer disc diffusion method. Nutrient agar plates were seeded with 24 h broth cultures of S. aureus, E. coli and Klebsiella sp. Sterile discs (10 mm diameter) impregnated with 25 µL of each fruit extract were placed on the seeded plates and incubated at 37 °C for 24 h. The diameter of the zone of inhibition around each disc was measured in millimetres and recorded.

2.6 Determination of Antioxidant Activity

Antioxidant activity was determined by the DPPH radical scavenging assay. One millilitre of each extract was mixed with 1 mL ethanol and 2 mL of 0.2 mM DPPH solution and incubated in the dark at room temperature for 30 min. Absorbance was recorded at 517 nm, with ascorbic acid (50–250 µg) as the positive control. Percentage DPPH scavenging activity was calculated as: % Scavenging = [(A0 − A1)/A0] × 100, where A0 is the absorbance of the control and A1 is the absorbance of the sample.

2.7 Statistical Presentation

Protease-activity values represent the mean of triplicate determinations. Antibacterial and antioxidant-activity values represent single determinations obtained under the assay conditions described above; formal inferential statistics (e.g., ANOVA, standard deviation) were not computed for these parameters and comparisons among samples are therefore presented descriptively.

3. RESULTS

3.1 Assay of Protease Activity

Table 1 presents the protease activity of bromelain, papain and actinidin extracted from pineapple, papaya and kiwi, respectively. Papain exhibited the highest activity (99.343 µmol/mL/min), which was nearly twice that of bromelain (55.190 µmol/mL/min) and approximately 18-fold that of actinidin (5.519 µmol/mL/min).

Table 1. Protease activity of bromelain, papain and actinidin extracted from pineapple, papaya and kiwi fruit samples.

S. No.	Sample (Protease)	Protease Activity (µmol/mL/min)
1	Bromelain (pineapple)	55.190
2	Papain (papaya)	99.343
3	Actinidin (kiwi)	5.519

Fig. 1. Comparison of protease activity of fruit samples

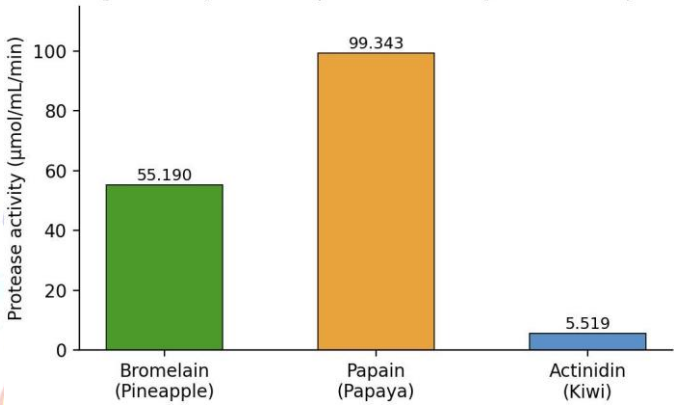

Fig. 1. Comparison of protease activity of fruit samples (Bromelain, Papain and Actinidin).

3.2 Antibacterial Activity

The zones of inhibition produced by pineapple, papaya and kiwi extracts against S. aureus, E. coli and Klebsiella sp. are shown in Table 2. Pineapple extract produced the largest zones of inhibition against all three organisms, followed by kiwi and papaya. All three extracts inhibited the Gram-positive organism (S. aureus) more effectively than the Gram-negative organisms (E. coli and Klebsiella sp.).

Table 2. Zone of inhibition (mm) produced by pineapple, papaya and kiwi extracts against Staphylococcus aureus, Escherichia coli and Klebsiella sp.

S. No.	Sample	*S. aureus* (mm)	*E. coli* (mm)	*Klebsiella sp.* (mm)
1	Pineapple	19	18	16–17
2	Papaya	14–15	10	10
3	Kiwi	14–15	12	14

Fig. 2. Comparison of antibacterial activity of fruit extracts against test organisms

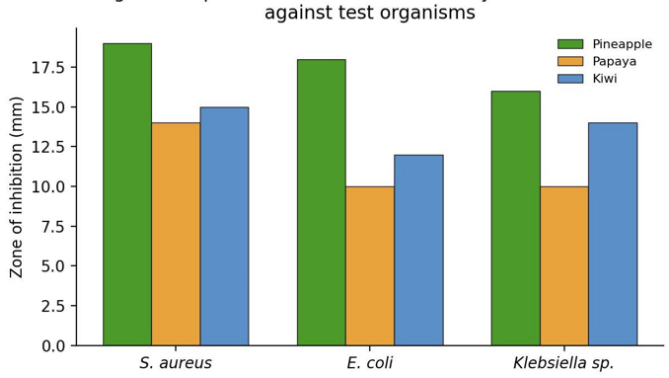

Fig. 2. Comparison of antibacterial activity of fruit extracts against test organisms.

3.3 Antioxidant Activity

Table 3 shows the percentage DPPH radical scavenging activity of the three fruit extracts. Pineapple extract exhibited the highest scavenging activity (83.02%), closely followed by papaya (81.77%), while kiwi showed comparatively lower activity (60.46%); all three extracts scavenged more than 50% of the DPPH radical.

Table 3. Percentage DPPH radical scavenging activity of pineapple, papaya and kiwi fruit extracts.

S. No.	Sample	DPPH Scavenging Activity (%)
1	Pineapple	83.02
2	Papaya	81.77
3	Kiwi	60.46

Fig. 3. Antioxidant activity of fruit extracts by DPPH method

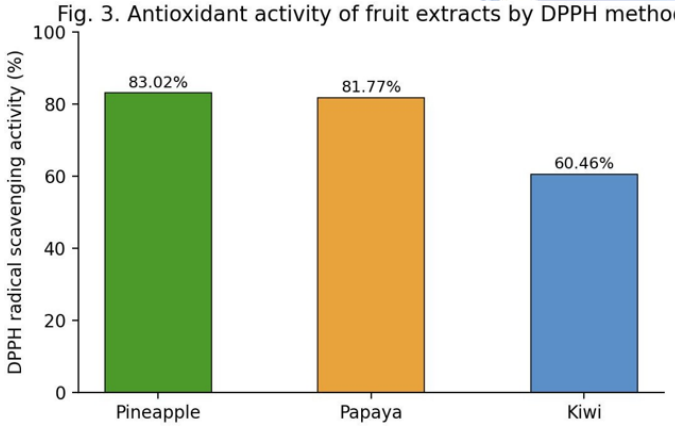

Fig. 3. Antioxidant activity of different fruit samples by the DPPH method.

4. DISCUSSION

The comparative assay of protease activity revealed that papain from papaya exhibited markedly higher activity than bromelain from pineapple and actinidin from kiwi. This finding is consistent with the well-documented proteolytic efficiency of papain and is in line with recent comparative kinetic studies confirming distinct hydrolytic behaviour among papain, bromelain and actinidin on protein substrates [18]. The relatively low activity of actinidin observed here may reflect its narrower substrate specificity, which has been reported to be more restricted towards globular proteins compared with papain.

The antibacterial screening showed that all three extracts inhibited the Gram-positive organism S. aureus more effectively than the Gram-negative organisms E. coli and Klebsiella sp., a pattern commonly attributed to the additional outer-membrane barrier of Gram-negative bacteria that limits the penetration of plant-derived antimicrobial compounds. Pineapple extract consistently produced the largest inhibition zones, which may be related to its protease activity, mild acidity and phenolic content, factors that have similarly been implicated in the antibacterial action of fermented pineapple-and papaya-derived preparations against both Gram-positive and Gram-negative pathogens [19]. The continuing relevance of plant-derived antibacterial agents against Gram-negative organisms such as E. coli has also been highlighted in more recent screening studies combining in vitro and computational approaches [22, 23].

The antioxidant assay indicated that pineapple and papaya extracts possessed comparable and considerably higher DPPH scavenging activity than kiwi. Fruits are known to be rich sources of ascorbic acid, phenolics, flavonoids and carotenoids, and the differences observed among the three extracts likely reflect variation in the concentration and composition of these antioxidant constituents.

Taken together, the protease, antibacterial and antioxidant profiles observed in this study suggest that pineapple, papaya and kiwi extracts each offer distinct but complementary bioactive properties, supporting their continued relevance in nutraceutical, pharmaceutical and food-preservation applications.

5. CONCLUSION

This comparative study demonstrates that papaya-derived papain possesses the highest protease activity among the three fruit extracts examined, while pineapple extract shows the strongest antibacterial and antioxidant activity. All three extracts were more inhibitory towards Gram-positive than Gram-negative

bacteria and retained substantial free-radical scavenging capacity. These findings support the potential of pineapple, papaya and kiwi fruit extracts as natural sources of proteolytic, antimicrobial and antioxidant agents for application in the food, pharmaceutical and cosmeceutical sectors. Further studies incorporating replicate-based statistical validation, phytochemical profiling and in vivo evaluation are recommended to substantiate these findings.

ACKNOWLEDGEMENTS

The authors thank the Department of Biochemistry, Yogi Vemana University, Kadapa, for providing the laboratory facilities used in this study.

Conflict of interest statement

Authors declare that they do not have any conflict of interest.

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