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Application of *Psidium guajava* Leaf Ethanol Extract Coating for Extending the Shelf Life of Indian Prawn (*Penaeus indicus*) Under Chilled Storage

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Abstract

The present study aimed to characterise the *Psidium guajava* leaf powder and its ethanolic extract and to analyse its preservative effect on Indian prawn (*Penaeus indicus*) under chilled storage. The dried *P. guajava* leaf powder contained high levels of fibre, ash, and carbohydrate, as well as high potassium and magnesium content. Antioxidant assays showed 97.40 ± 0.03% (DPPH assay) and 78.20 ± 0.01% (ABTS assay) inhibition with a 20% ethanolic extract, attributable to its total phenolic and flavonoid content. An antimicrobial study revealed that the ethanolic extract at 20% concentration showed strong inhibitory zones against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (*MRSA)* (34.00 ± 0.01 mm), *S. aureus* (27.00 ± 3.00 mm), and *Bacillus cereus* (17.00 ± 2.00 mm), but did not exhibit antimicrobial activity against the tested Gram-negative bacteria. During the storage study, biochemical, microbiological, and sensory qualities of *P. guajava* leaf extract-coated *P. indicus* were evaluated. In biochemical analysis, TBARS values revealed that the samples treated with 20% and 40% concentration had better shelf life. Similarly, TVB-N and TMA values showed that 20% and 40% treated samples exceeded the acceptable limit on the 18th day of storage, whereas the control sample exceeded the limit on the 12th day. Microbiological analysis demonstrated that 20% and 40% coatings effectively inhibited psychrotrophic and mesophilic bacterial growth and exceeded the acceptable limit on the 18th day of storage, whereas the control sample exceeded the acceptable limit on the 12th day. Overall, *P. guajava* leaf extract coatings, especially at 20% and 40%, markedly enhanced the shelf life of *P. indicus* during chilled storage and could be used as a practical natural preservative.

Keywords: *Psidium guajava, Penaeus indicus,* edible coating

Introduction

Penaeus indicus is a popular prawn and is valued globally for both its commercial importance and nutritional benefits. It is widely consumed and extensively distributed across regions such as India, Africa, Indonesia, and China (Ahamed, Ahmed, & Ohtomi, 2022). In 2024, India exported 7.16 lakh tonnes of frozen shrimp to various nations, with *P. indicus* accounting for a significant share of this total (MPEDA, 2024). Nutritionally, they are rich in high-quality protein, vitamin B1 and B2, and minerals such as zinc (Zn), sodium (Na), calcium (Ca), and potassium (K) (Abdel-Salam, 2013). In terms of calories, they are low due to their reduced carbohydrate and fat content, but they have high cholesterol levels. The bottom-dwelling trait of prawns leads to higher bacterial loads at the time of catch (Bindu, Ginson, Kamalakanth, Asha, & Gopal, 2013). Due to their high nutritional value and microbial load, preserving prawn quality while maintaining its natural microbial balance remains a significant challenge for seafood processors. They spoil due to the presence of free amino acids, bacterial activity, and muscle fibre degradation, which produce off-flavours and odours, and lead to the formation of substances such as hydrogen sulfide (H_2S), ammonia (NH_3), and volatile metabolites (Gennari & Tomaselli, 1988). Therefore, seafood

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processors often use chemical preservatives to uphold prawn quality and meet international market demands. However, these compounds may pose health risks when consumed with prawns. Furthermore, consumer awareness regarding the health and nutritional benefits of fish and fishery products has compelled the industries to produce these items with minimal or no chemical preservatives. As a result, the use of natural preservatives to maintain the quality of fish and fishery products is gaining popularity as a safer alternative (Sabu, Xavier, & Sasidharan, 2021).

Natural preservatives are sourced from plants, animals, or microbes, which provide a safer and often more consumer-friendly alternative to chemical preservatives. They mainly extend shelf life and improve food safety through their functional properties, such as antioxidant and antimicrobial effects. They also offer benefits such as boosting the nutritional value and sensory qualities of food. Overall, natural preservatives are considered safer, more sustainable, and more environmentally friendly than many artificial preservatives. Plant-based natural preservatives are frequently used in food processing because of their antioxidant and antibacterial properties, as well as their enhanced sensory attributes (Oulahal & Degraeve, 2022).

P. guajava is a widely cultivated plant worldwide, valued for its fruits and leaves due to their diverse medicinal and nutritional qualities (Naseer, Hussain, Naeem, Pervaiz, & Rahman, 2018). The fruit contains health-promoting nutrients, including protein, carbohydrates, and minerals (Khanna, Singh, Chauhan, & Chauhan, 2025). The leaves offer various potential health benefits because of their rich nutritional content and bioactive compounds. They are recognized for their antioxidant, antimicrobial, anti-inflammatory, antidiarrheal, antispasmodic, cough-sedative, antihypertensive, anti-obesity, and antidiabetic properties (Chen & Yen, 2007). These leaves can help manage diabetes, improve cardiovascular health, treat specific respiratory and gastrointestinal ailments, increase platelet counts in patients with dengue fever, and support skin and hair care. Several studies on animal models have also shown that the leaf extracts are powerful antitumor, anticancer, and cytotoxic agents (Ashraf, Sarfraz, Rashid, & Shahid, 2015). These bioactive compounds can be extracted using various solvents, including ethanol, acetone, and methanol.

The bioactive compounds in the leaf, such as ferulic acid and phenolic acids, exhibit excellent antioxidant activity (Chen & Yen, 2007). Water-soluble tannins and flavonoid compounds, such as quercetin, and other phytochemicals demonstrate bacteriostatic effects against specific foodborne pathogens (Widsten, Cruz, Fletcher, Pajak, & McGhie, 2014; Martinengo, Arunachalam, & Shi, 2021). Considering its health benefits and nutritional composition, it has been utilised as a functional food ingredient. Some researchers have used it to fortify pork sausages (Tran et al., 2020), and is also used as an herbal tea (Lilachjini & Haroon, 2023). Limited studies have investigated the application of *P. guajava* leaf extract in food preservation, particularly in seafood systems. Therefore, this study aimed to characterise *P. guajava* leaf extract and evaluate its preservative effect on *P. indicus* during chilled storage.

Materials and Methods

Fresh *P. guajava* leaves were collected, thoroughly washed, packed in zip-lock pouches, and transported to the laboratory for further processing. The leaves were dried in a mechanical dryer at 50 °C for 48 hours to ensure proper drying. After drying, the leaves were ground into powder using a food processor. Ten grams of powder were used in the extraction process, which was performed with ethanol-distilled water solutions at different concentrations (10-80%) using a magnetic stirrer for 4 hours. The extracted solutions were then filtered through Whatman No. 1 filter paper. Finally, the ethanol was removed using a rotary evaporator at 45 °C under a 100 mbar vacuum, and the *P. guajava* leaf extract was stored for further studies.

Proximate composition and mineral content of *P. guajava* leaf powder were determined using AOAC (2024) methods. Moisture and ash were measured by gravimetric methods. Protein content was determined using the Kjeldahl method, fat by Soxhlet extraction, fibre by the enzymatic-gravimetric method, and carbohydrates by the anthrone-sulfuric acid method. Mineral composition was analysed using atomic absorption spectrometry (AAS).

Colour analysis was performed using a Hunter Lab colourimeter. Based on the parameters such as L* (lightness), a* (red-green), and b* (yellow-blue) values, the colour of *P. guajava* leaf powder and extract was described.

The phytic acid content of *P. guajava* leaf powder was analysed using the method described by Ola and Oboh (2000), with some modifications. This involved soaking 2 g of powder in 2% HCl for 3 h before filtration. Afterwards, 25 mL of the filtrate, 5 mL of 0.3% NH_4SCN, and 53 mL of distilled water were combined. The filtrate was then tested against a 0.01 M $FeCl_3$ standard until a brownish tint persisted for 5 seconds. The amount of phytic acid was calculated by multiplying the phytin phosphorus value by 3.55.

Oxalate content was determined using a modified version of the Krishna and Ranjhan (1980) method. Two grams of cake residue were digested in a 250 mL flask with 10 mL 6 M HCl and 190 mL distilled water in a boiling water bath for 1 h. After cooling, the mixture was filtered. To 50 mL of filtrate, 20 mL of 6 M HCl was added, evaporated to half volume, and filtered again. The residue was rinsed with warm distilled water, and 25 mL of filtrate was titrated with 0.1 M $KMnO_4$ to a faint pink endpoint (30 s) after adding three drops of methyl orange. Total oxalate = TV × 0.0045, where TV is titrant volume.

Hydrocyanic acid (cyanide) content was determined by the AOAC (2024) method. Five grams of powder were mixed with 50 mL of distilled water in a flask, stored for 24 hours, and filtered. To the filtrate, 1–4 mL alkaline picrate and 1 mL 0.1 M NaOH were added. The tube was corked, incubated at 25 °C in a water bath for 5 minutes, cooled, and the absorbance was read at 490 nm against a blank.

Tannin content was determined using the method of Allen, Grimshaw, Parkinson, and Quarmby (1974) with minor modifications. Briefly, about 0–5 g of the cake residue was transferred to a 100 mL bottle. Then, 50 mL of distilled water was added, and the mixture was shaken vigorously for 1 hour using a mechanical shaker. After filtering the solution into a 50 mL volumetric flask, the required volume was measured. A test tube was filled with 5 mL of the filtrate, 2 mL of 0.1 M $FeCl_3$ in 0.1 N HCl, and 0.008 M potassium ferrocyanide. The spectrophotometer was used to measure the absorbance at a wavelength of 725 nm.

DPPH activity was assessed following Jadid et al. (2017) with slight modifications. A DPPH solution in ethanol (2 mL) was mixed with 2 mL of extract at various concentrations. Ethanol served as the control, and ascorbic acid as the standard. Absorbance was measured at 517 nm using a spectrophotometer. All tests were done in triplicate, and activity was calculated using the following equation.

$$\% \text{ inhibition} = \frac{\text{Absorbance of the control} - \text{Absorbance of sample}}{\text{Absorbance of the control}} \times 100$$

The $ABTS^+$ reagent was prepared 16 hours before use (Kaur, Dhull, Sandhu, Salar, & Purewal, 2018) by dissolving $K_2S_2O_8$ (33 mg) and $ABTS^+$ (17.2 mg) in 5 mL distilled water (x2), and incubating in the dark for 16 h. The solution was diluted with double-distilled water (1:60). Extracts (100 μL) were reacted with $ABTS^+$ reagent (3 mL) in amber vials in the dark for 10 minutes. Absorbance was measured at 732 nm with a negative control. Results were expressed as % $ABTS^+$ scavenging activity at 100 μg/mL.

$$\text{Discolouration activity (\%)} = \frac{\text{Absorbance of the control} - \text{Absorbance of sample}}{\text{Absorbance of the control}} \times 100$$

The Folin-Ciocalteu method was used to estimate the total phenolic content of *P. guajava* (Chiari et al., 2012). Briefly, a 25 mL standard flask was filled with 1 mL of extract of various concentrations, diluted with 9 mL of distilled water. The mixture was then vortexed with 1 mL of Folin-Ciocalteu reagent. To reach the 25 mL mark, 5 mL of 7% Na_2CO_3 was added to the flask after 5 minutes, and then it was diluted with deionised water. Finally, a spectrophotometer measured the absorbance at 750 nm after incubating the flask for 90 minutes. The results were expressed in gallic acid equivalents (mg GAE g^{-1}).

Total flavonoid content (TFC) was determined by the aluminium chloride colourimetric method (Shraim, Ahmed, Rahman, & Hijji, 2021) using quercetin for calibration. Quercetin (10 mg) was dissolved in 80% ethanol to obtain 25, 50, and 100 μg/mL standards. For each, 1.5 mL 95% ethanol, 0.5 mL standard, 0.1 mL 1 M aluminium chloride, 0.1 mL potassium acetate, and 2.8 mL distilled water were mixed. After 30 min incubation at room temperature, absorbance was read at 415 nm (Shimadzu UV-160A). Blanks replaced 10% aluminium chloride with water. The same procedure was applied to 0.5 mL ethanol extracts or 15 flavonoid standards (100 ppm). Results were expressed as mg quercetin equivalents per gram extract (mg QE/g):

TFC (mg QE/g) = (C × V) / m

where C = flavonoid concentration from calibration curve (mg/mL), V = total extract volume (mL), m = sample mass (g).

For the well diffusion method, 10 mm-wide wells were created in MHA plates, and 0.1 mL of bacterial inocula, including methicillin-resistant *S. aureus* (*MRSA), B. cereus, S. aureus, Vibrio cholerae*, and *Salmonella enteritidis* (10^7 CFU/mL), was carefully swabbed onto them. Subsequently, 100 µL of the extract samples was added to the wells. Furthermore, the plates were incubated at 37 °C for 24 hours. After incubation, the zone of inhibition was measured using a Halos calliper (Mirtaghi, Nejad, Mazandarani, Livani, & Bagheri, 2016).

For the preservative study, medium-sized fresh *P. indicus* were obtained from the Kalamukku fish market in Kochi, Kerala, and transported to the laboratory on ice. In the laboratory, they were washed thoroughly and prepared into peeled, deveined (PUD) form. Then, they were treated with 10% to 40% ethanol extract of *P. guajava* leaves for 10 minutes. Finally, the excess extract was drained off, and the prawn samples were packed in sterile zip-lock plastic pouches and stored at 4 °C. During storage, biochemical, microbiological, and sensory characteristics were analysed.

The oxidative stability of prawn samples was evaluated by measuring TBARS values using the method of Tarladgis, Watts, Younathan, and Dugan (1960), with slight modifications. In brief, 10 g of the macerated fish sample was treated with 2% HCl (100 mL) and then placed in the distillation unit for distillation. In the distillation units, 50 mL of distillate was collected. Additionally, 5 mL of distillate and 5 mL of TBA reagent were added to a test tube, with 5 mL of distilled water serving as a blank in place of the distillate. After immersing the test tubes in boiling water for 30 minutes, the absorbance at 510 nm was measured. The results were expressed as milligrams of malonaldehyde per kilogram of the sample.

Conway diffusion method was used to measure TMA and TVB-N in prawn samples (Ali, Hasan, Moyaduzzaman, & Faruque, 2008). Briefly, 4 g of the sample was ground with 16 mL trichloroacetic acid (TCA) using a mortar and pestle. A 1% boric acid solution with bromocresol green and methyl red indicators was placed in the inner chamber of the Conway unit. The inner and outer rings received 1 mL each of the sample and saturated potassium carbonate for TMA-N and TVB-N analysis. For TMA-N, 1 mL of 10% formaldehyde was added to the outer ring and mixed. The mixture was incubated at 37 °C for 1 hour, followed by titration in the inner ring with 0.02 N HCl. TVB-N was expressed as mg nitrogen per 100 g sample.

Briefly, 5 g of the prawn sample was placed in a beaker containing 25 mL of distilled water and mixed thoroughly. Afterwards, pH was recorded using the digital pH meter (Susanto, Agustini, Ritanto, Dewi, & Swastawati, 2011).

Plate counts for mesophilic and psychrotrophic organisms were conducted using the standard AOAC (2024) method. Briefly, a stomacher was used to aseptically homogenise 25 g of samples with 225 mL of sterile phosphate buffer. The resulting suspensions (1 mL) were serially diluted in test tubes containing 9 mL of sterile phosphate buffer and thoroughly mixed using a vortex mixer. Neogen Petrifilms USA provided 1 mL of test solution on Petri films (6400 Aerobic counts, 2 × 50), which was carefully inoculated. The incubation period was 96 hours at 4 °C for psychrotrophic bacteria and 24 hours at 37 °C for mesophilic bacteria. The bacterial colonies were then counted, and the average log-transformed counts were used to represent the results (log CFU/g).

Sensory evaluation was performed with semi-trained panellists. After boiling the *P. indicus* sample in a 1.5% sodium chloride solution for 10 minutes, the samples were assessed by the panellists for overall acceptability. Scoring was based on a nine-point hedonic scale as described by Meilgaard, Civille, and Carr (1999). Characteristics such as colour, appearance, texture, odour, and flavour were evaluated. An overall acceptance score was derived by averaging all individual scores. A sensory score of 6 was used as the threshold for acceptability.

The experimental results were expressed as mean ± standard deviation (n = 3). Statistical differences among treatments were analysed using one-way analysis of variance (ANOVA), followed by Tukey's HSD multiple comparisons test in IBM SPSS Statistics 25 (SPSS Inc., Chicago, IL, USA), with significance set at $p < 0.05$.

Results and Discussion

The proximate composition of *P. guajava* leaf dry powder and mineral analysis is given in Table 1. The moisture content of the dried powder was 2.70 ± 0.50%, and the protein content was 12.00 ± 0.25%. The fat percentage is very low compared to other components (3.50 ± 0.67%). *P. guajava* leaves are an abundant source of ash and fibre, indicating the presence of minerals and promoting dietary fibre intake when consumed. The carbohydrate content (26.30 ± 0.06%) was comparable with the value reported by Pawar et al. (2024), which is 38.23%. Overall, the proximate composition values suggest moderate levels of ash, fibre, and carbohydrates, along with low levels of fat and moisture, consistent with a previous report by Kumar et al. (2021).

The mineral composition of *P. guajava* leaves is shown in Table 2. It contains a high level of potassium (3915 ± 92.56 ppm), which exceeds that of other minerals in these leaves. Calcium (3083 ± 72.80 ppm) is the second most abundant element and can also serve as a dietary source of calcium (Gurusamy et al., 2020). Magnesium ranks third among the main minerals, supporting various bodily functions, including energy metabolism and DNA synthesis (Fatima et al., 2024). Manganese is another important element in *P. guajava* (452.30 ± 16.67 ppm), playing a crucial role in bodily functions by acting as a cofactor for many enzymes (Khoshru et al., 2023). The leaves also contain significant amounts of sodium (48.35 ± 9.51 ppm) and iron (157.40 ± 5.41 ppm). Overall, the mineral profile indicates that the leaves are rich in essential nutrients for bio-functional activities.

Colour & pH analysis are depicted in Table 2. The lightness (L*) value of leaf powder was 49.89 ± 0.13, indicating a relatively lighter colour compared to the extract, which exhibits a much lower lightness value of 14.80 ± 0.05. This notable decrease in lightness indicates a higher concentration of pigments in the extract, resulting in a darker appearance (Yue, Wang, & Yang, 2021). The a* values of (greenness/redness) leaf powder showed that the leaf powder had a stronger green tone (-2.8) than the extract (-0.06). This reduction can also be attributed to the oxidation of chlorophyll lost during extraction. Regarding the yellowness-blue coordinate (b*), the leaf powder showed a higher yellowness value (20.37 ± 0.28) than the extract (4.06 ± 0.04). This colour change might be due to the partial solubilization of flavonoids (Li et al., 2021). This also indicates that ethanol extraction is sufficient for *P. guajava*.

Table 1. Proximate composition and mineral analysis of *P. guajava* leaf powder

Proximate composition	Value (%)	Element	Value (ppm)
Moisture	2.70 ± 0.50	Ca	3083.00 ± 72.80
Protein	12.00 ± 0.25	Fe	157.40 ± 5.41
Fat	3.50 ± 0.67	K	3915.00 ± 92.56
Fibre	27.50 ± 0.45	Mg	2977.00 ± 148.00
Ash	27.00 ± 0.02	Mn	452.30 ± 16.67
Carbohydrate	26.30 ± 0.06	Na	48.35 ± 9.51

Results are expressed as mean ± standard deviation (n=3)

Table 2. Colour and pHof *P. guajava* leaf powderand extract

Sample	L*	a*	b*
Colour of leaf powder	49.89 ± 0.13	-2.85 ± 0.04	20.37 ± 0.28
Colour of leaf extract	14.8 ± 0.05	-0.06 ± 0.02	4.06 ± 0.04
pH of the extract (100%)	4.3 ± 0.05		

Results are expressed as mean ± standard deviation (n=3).

L* = lightness, a* = greenness [-ve]/redness [+ve], b* = yellowness/blueness [-ve]).

Antinutritional factors, including tannins, phytic acid, oxalate, and cyanide content, are listed in Table 3. Among them, tannin (0.02 mg/100 g) is the highest component, followed by oxalate (1.54 ± 0.01 mg/100 g) and phytate (1.00 ± 0.08 mg/100 g). Cyanide is not detected in the sample. Generally, standard processing techniques such as dry and wet heating, solvent extraction, and enzyme treatment reduce the harmful effects of antinutrients in plant-based materials (Natesh et al., 2017).

The DPPH and ABTS antioxidant activities of *P. guajava* leaf ethanol extract at various concentrations is shown in Table 4. The "% inhibition" in the DPPH assay increases with higher concentrations and reaches a maximum at 20% concentration (97.40 ± 0.03%). Similarly, the ABTS assay shows the highest activity at 25% (80.00 ± 0.01%). The antioxidant activities of *P. guajava* ethanol extract are attributed to its polyphenol and flavonoid content (Braga et al., 2014). Saturation occurs with DPPH at 20%, but at 25% concentration, a slight decrease in DPPH chelating activity was observed due to the plateau effect.

Table 3. Anti-nutritional factors of *P. guajava dry* leaf *powder*

Anti-nutritional factors	mg/100 g
Tannin	2.00 ± 0.02
Phytate	1.00 ± 0.08
Oxalate	1.54 ± 0.01
Cyanide	ND

Values represent mean ± standard deviation (SD) from triplicate analyses (n=3)

Table 4. Antioxidant activity of *P. guajava* leaf extract

P. guajava leaf extract (Concentration in %)	DPPH (% Inhibition)	ABTS (% Inhibition)
5%	37.40 ± 0.01^{a}	28.00 ± 0.01^{a}
10%	42.20 ± 0.02^{b}	41.00 ± 0.01^{b}
15%	68.00 ± 0.01^{c}	67.00 ± 0.01^{c}
20%	97.40 ± 0.03^{d}	78.20 ± 0.01^{d}
25%	97.20 ± 0.02^{d}	80.00 ± 0.01^{e}

Values are presented as mean ± standard deviation (n = 3).

Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$) based on one-way ANOVA followed by Tukey's HSD test.

Table 5. Total phenolic and flavonoid contentof *P. guajava* leaf extract

P. guajava leaf extract (Concentration in %)	Total phenols (mg GAE g^{-1}).	Flavonoids (mg QE/g extract)
10%	20.50 ± 1.50^{a}	15.50 ± 0.50^{a}
20%	33.70 ± 1.40^{b}	19.50 ± 0.90^{b}
30%	35.20 ± 1.50^{b}	22.10 ± 1.20^{c}
40%	46.50 ± 1.60^{c}	28.90 ± 1.10^{d}

Results are expressed as mean ± standard deviation (n = 3). Values with different superscript letters in the same column are significantly different ($p < 0.05$) as determined by one-way ANOVA with Tukey's HSD post hoc test.

The presence of active compounds like phenols and flavonoids was shown in Table 5. The total phenol and flavonoid content showed an increasing trend with increasing concentrations. It reached 46.50 ± 1.60 mg GAE g^{-1} at the 40% level, while flavonoids peaked at 40% concentration with a value of 28.90 ± 1.10 mg QE/g extract. Polyphenols and flavonoids contribute to antioxidant activity by scavenging free radicals and inhibiting lipid oxidation. In this study, total polyphenols react with free radicals, thereby slowing lipid oxidation and delaying rancidity (Duthie, 1999), as observed in antioxidant studies.

Table 6 shows the zones of inhibition for various concentrations of *P. guajava* leaf extract against five bacterial strains, as determined by agar well diffusion assays. At a 10% concentration, *B. cereus* exhibited an inhibition zone of 10.00 ± 1.00 mm, while the rest showed no zone. The 20% extract concentration exhibited the largest zone of inhibition against *MRSA* at 34.00 ± 0.00 mm, followed by *S. aureus* (27.00 ± 4.00 mm) and *B. cereus* (17.00 ± 1.00 mm), which are causative agents of atopic dermatitis and food poisoning, respectively. Additionally, concentrations of 30% and 40% did not show statistically significant differences ($p < 0.05$) compared to the 20% concentration. The antimicrobial activities of *P. guajava* leaf extract are due to the presence of antimicrobial compounds such as 2,4-di-tert-butylphenol, caryophyllene oxide, and γ-murolene. A study by Biswas, Rogers, McLaughlin, Daniels, and Yadav (2013) reported that methanolic and ethanolic leaf extracts of guava exhibited the highest inhibitory activity against foodborne and spoilage gram-positive bacteria but showed no antimicrobial effect against gram-negative

Table 6. Antimicrobial activity of *P. guajava* leaf extract

P. guajava leaf extract (Concentration in %)	Zone of Inhibition (mm)				
	MRSA	*Bacillus cereus*	*Staphylococcus aureus*	*Vibrio cholerae*	*Salmonella enteritidis*
10%	ND	10.00 ± 1.00[a]	ND	ND	ND
20%	34.00 ± 0.01[a]	17.00 ± 2.00[b]	27.00 ± 3.00[a]	ND	ND
30%	32.00 ± 0.01[b]	17.00 ± 2.00[b]	21.00 ± 1.00[b]	ND	ND
40%	30.00 ± 0.02[c]	17.00 ± 1.00[b]	23.00 ± 0.01[b]	ND	ND
Control (ethanol)	ND	ND	ND	ND	ND

Data are reported as mean ± standard deviation (n = 3); different superscript letters within each column indicate significant differences ($p < 0.05$) using one-way ANOVA followed by Tukey's HSD test, and ND indicates no detectable inhibition zone.

organisms. A similar result was observed in our study, which showed no inhibition zone against Gram-negative bacteria.

The TBARS value measures lipid oxidation in fish and fishery products (Connell & Howgate, 1986). The initial TBARS value was 0.34 ± 0.03 mg MDA/kg, indicating that prawns were fresh (Fig. 1A). As storage days increased, the TBARS values rose due to oxidation. The control (C) sample exceeded the acceptable limit of 2 mg MDA/kg on the 9th day of storage (2.00 ± 0.03 mg MDA/kg). Samples treated with 10% and 30% *P. guajava* leaf extracts exceeded the limit on 12 days, with values of 2.90 ± 0.02 and 2.50 ± 0.03 mg MDA/kg, respectively. While *P. guajava* leaf extract-treated 20% and 40% samples remained within acceptable limits on the 15th day, recording values of 2.00 ± 0.01 and 2.20 ± 0.01 mg MDA/kg, respectively. This shows that the presence of antioxidant and antimicrobial compounds, such as flavonoids and polyphenols, in guava leaf extract effectively minimizes lipid oxidation during chilled storage of prawns.

TMA is directly linked to the development of "fishy" odours and is considered a sensitive spoilage marker. Values above 10–15 mg/100 g indicate spoilage in shrimp (Nevigato, Masci, Casini, Caproni, & Orban, 2018). Changes in the TMA values of coated samples during chilled storage are shown in Fig. 1B. The TMA levels of the 40% and 20% coated samples surpassed the accepted limit on the 15th day of storage. The values of the control sample rapidly exceeded the acceptable limit on the 9th day, reaching 15.50 ± 1.50 mg per 100 g. The samples treated with 10% and 30% leaf extract exceeded the acceptable limit on the 12th day with values of 18.00 ± 0.70 mg per 100 g and 15.13 ± 0.00 mg per 100 g, respectively. This aligns with findings that TMA levels increase rapidly with bacterial activity, indicating rapid quality deterioration, which suggests that the 20% and 40% coatings are the most suitable for prawn storage.

Parallel changes were observed in Total Volatile Basic Nitrogen (TVB-N) values, which is a comprehensive spoilage index encompassing TMA, dimethylamine, and ammonia. TVB-N values above 30–35 mg/100 g are generally considered a spoilage threshold for iced fish (Hopkins, Holman, Bekhit, & Giteru, 2020). The TVB-N levels during storage are charted in Fig. 1C. A similar trend was observed with the 20% and 40% coated samples crossing the spoilage threshold after 15th day of storage, reaching values of 40.00 ± 1.50 mg/100 g and 40.00 ± 1.20 mg/100 g, respectively. However, the control samples reached spoilage limit on the 6th day with a value of 40 ± 0.0 mg/100 g. Meanwhile, the 10% and 30% coated samples exceeded their limits on the 12th day, with values of 35.00 ± 0.80 mg/100 g and 35.50 ± 0.30 mg/100 g, respectively. The TVBN levels in seafood indicate microbial spoilage related to amino acid decarboxylation, which correlates with mesophilic and psychrophilic plate count changes over storage days.

The initial pH (Fig. 1D) of the prawn sample was 6.80 ± 0.30. When the storage progressed, the pH of the control sample increased rapidly, reaching 7.30 ± 0.50 by day 3 and exceeding 8.00 ± 0.30 by day 6, before stabilising at 8.50 ± 0.20 from day 9 to day 15. Extract-coated samples displayed varied patterns

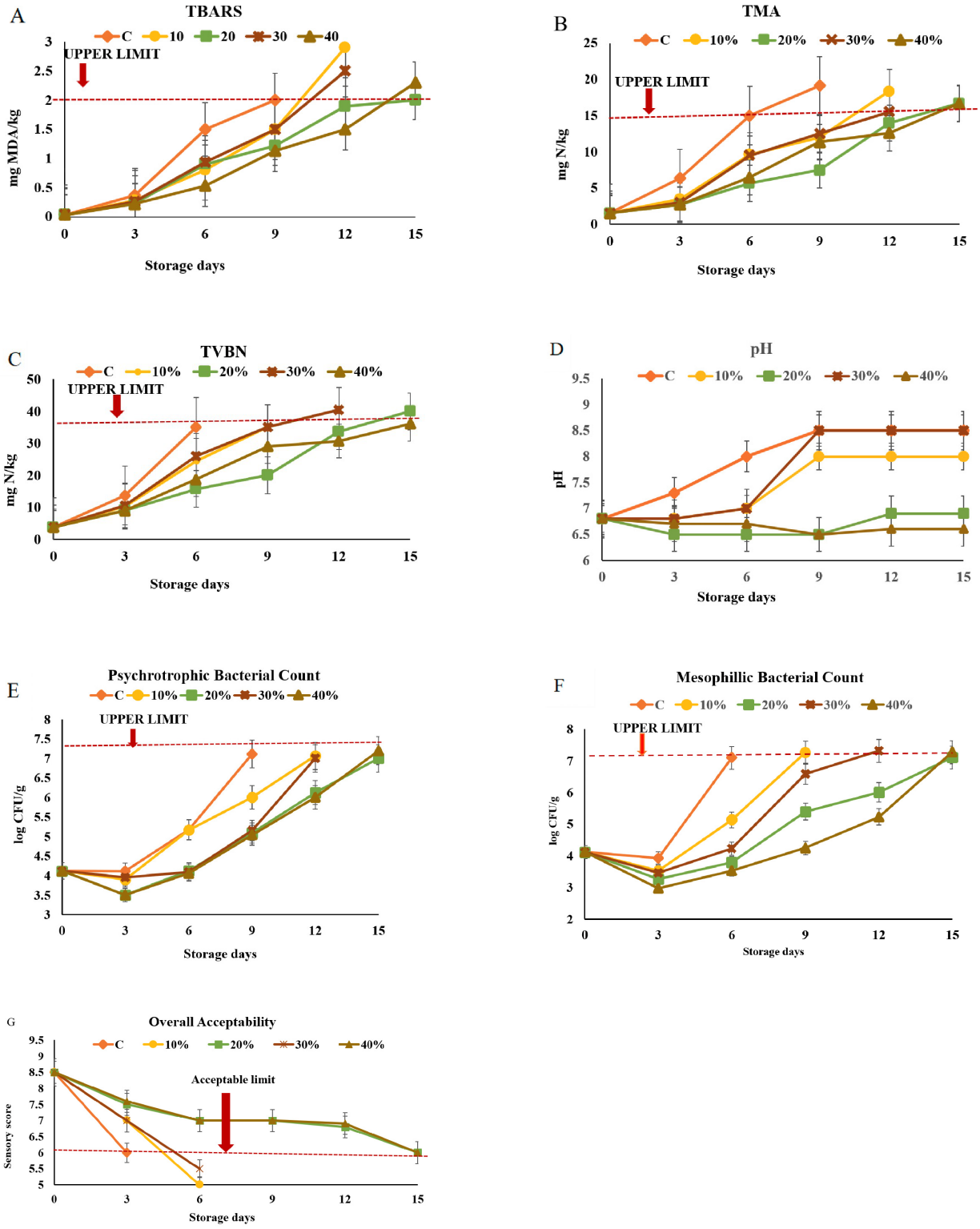

Fig. 1. Quality characteristics of *Pisidium guajava* ethanol extract-coated *Penaeus indicus* during chilled storage (A) TBARS (B) TMA (C) TVBN (D) pH (E) Psychrotrophic Bacterial Count (F) Mesophilic Bacterial Count (G) Overall Acceptability

of pH change depending on the concentration used. Samples coated with 10% extract maintained an initial pH of 6.80 ± 0.30 for 3 days, then increased moderately to 7.0 on day 6 and rose sharply to 8.00 ± 0.40 by day 9, where it remained until day 15. The 30% extract group showed a similar rising trend as the control, reaching a pH of 8.50 ± 0.30 by day 9. In contrast, samples treated with 20% and 40% extracts exhibited a significant delay in pH increase. The 20% extract-treated samples had pH values below 7.0 ± 0.0 up to 12 days and reached 7.57 ± 0.10 on day 15. The 40% extract group demonstrated the most stable pH profile, staying close to the baseline up to day 15 and reaching 7.00 ± 0.20 after the storage period.

Changes in psychrotrophic bacterial counts are a key indicator of fish spoilage under chilled conditions (Mol, Erkan, Üçok, & Tosun, 2007). Psychrotrophic counts of extract-treated samples during chilled storage are shown in Fig. 1E. The initial count of the sample was 4.11 ± 0.02 log CFU/g, indicating the freshness of the prawn samples. During storage, the counts steadily increased in all samples. Till the 3rd day of storage, the count decreased due to the application of extracts, which may have limited bacterial growth due to antimicrobial effects. After the 3rd day, the bacterial count began to rise significantly throughout the storage period. The control samples exceeded the acceptable limit of 7 log CFU/g on the 9th day of storage (8.71 ± 0.01 log CFU/g). Samples coated with 10% and 30% extract exceeded this limit on the 12th day of storage, with the value of 7.27 ± 0.02 log CFU/g and 7.00 ± 0.02 log CFU/g, respectively. However, samples coated with 20% and 40% extract surpassed the limit on the 15th day of storage, with counts of 7.40 ± 0.02 log CFU/g and 7.00 ± 0.02 log CFU/g, respectively. This demonstrates the effectiveness of the 20% and 40% coating solutions against bacterial growth during chilled storage.

The mesophilic bacterial count of *P. guajava* leaf extracts treated prawn samples is shown in Fig. 1F. The initial bacterial count was 4.11 log CFU/g, with an upward trend observed in all samples throughout storage. On the 3rd day, the mesophilic counts decreased in the treated samples due to the application of extracts, which retarded the bacterial growth for a while. After that, bacterial growth gradually rose as the storage period increased. The control sample exceeded the overall acceptance limit on the 6th day. Meanwhile, the samples coated with 40% and 20% coatings surpassed the limit on the 15th day with values (7.10 ± 0.02 log CFU/g) and (7.30 ± 0.02 log CFU/g), respectively. The other coated samples, with 30% and 10% coatings, exceeded the spoilage limit on the 12th day (7.00 ± 0.02 log CFU/g) and the 9th day (7.2 ± 0.01 log CFU/g), respectively. Overall, it has been demonstrated that *P. guajava* leaf extracts effectively preserve the prawn during chilled storage, particularly at concentrations of 20% and 40%.

The overall acceptance of prawns coated with *P. guajava* leaf extracts at various concentrations under chilled conditions is shown in Fig. 1G. All samples experienced a significant, gradual decline in sensory scores during storage ($p < 0.05$). The initial sensory score was 8.50 ± 0.01, and noticeable differences between the samples appeared from the third day of storage. Colour changes and odour development in the control samples by the 3rd day caused them to exceed the acceptable limit. Similarly, 10% and 30% coated samples exceeded the limit on the sixth day of storage. Conversely, after 15 days of chilled storage, the samples coated with 20% and 40% extracts exceeded the acceptable limit on the 15th day (5.00 ± 0.01 and 5.05 ± 0.01, respectively). These results correlated with changes in the microbial and biochemical properties of the prawn samples, indicating that *P. guajava* extracts could serve as an effective primary edible coating to extend the shelf life during chilled storage.

In vitro studies confirmed the superior antioxidant and antimicrobial activities of the 20% *P. guajava* leaf ethanol extract, while the 40% extract showed the highest polyphenol and flavonoid content, which contributes to its preservative effects. Statistical analysis of the experimental data was performed using one-way analysis of variance (ANOVA), followed by Tukey's HSD post hoc test, which revealed significant differences ($p < 0.05$) among treatments. When applied as a coating to Indian prawn (*P. indicus*) during chilled storage, both 20% and 40% extracts demonstrated similar effectiveness in delaying spoilage and microbial growth across biochemical and microbiological quality parameters, significantly outperforming other concentrations, including 30%, 10%, and the control. These findings highlight the potential of *P. guajava* leaf extract as a natural preservative in edible coating systems. Furthermore, this eco-friendly approach reduces reliance on synthetic chemical preservatives and supports the development of biodegradable edible

coatings, thereby helping reduce plastic pollution in food packaging.

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