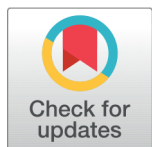


ORIGINAL ARTICLE



OPEN ACCESS

Received: 26/07/2026

Accepted: 13/08/2026

Published: 05/09/2026

Citation: Kiruthiga Periyannan, Balachandar Subbu, Hemamala Selvaraj (2026) Microbial Diversity of Spoiled Fruits and Vegetables and Application of Chitosan–Cellulose–Green Tea Biocomposite Coating for Postharvest Preservation. Indian Journal of Science and Technology 19(31): 2217-2226. <https://doi.org/10.17485/IJST/V19i31.1102>

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Funding: None

Competing Interests: None

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Published By Indian Society for Education and Environment ([iSee](#))

ISSN

Print: 0974-6846

Electronic: 0974-5645

Microbial Diversity of Spoiled Fruits and Vegetables and Application of Chitosan–Cellulose–Green Tea Biocomposite Coating for Postharvest Preservation

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Abstract

Objectives: To characterise the microbial diversity associated with spoiled fruits and vegetables and to design a multifunctional biocomposite coating based on chitosan, cellulose and green tea extract for postharvest preservation and shelf-life extension. **Method:** Spoiled samples of Tomato, Cucumber, banana, and Grape were gathered from Sulur Market, Coimbatore, Tamil Nadu, India during the winter season (December 2025). Microbial diversity was determined by serial dilution, selective culture medium, Gram staining and biochemical assays. Chitosan was isolated from the waste of prawn shells and characterised by FTIR spectroscopy. Antioxidant activity was assessed by DPPH assay. Antibacterial activity was evaluated by agar well diffusion method. Fresh fruits and vegetables were coated with a chitosan–cellulose (coir pith)–greentea extract biocomposite and compared with uncoated controls. **Findings:** Spoilage bacteria consisted of *Bacillus*, *Staphylococcus*, *Pseudomonas*, *Enterobacter*, *Klebsiella*, *Escherichiacoli*, *Salmonella*, *Vibrio* and *Enterococcus* spp. The FTIR analysis showed successful chitosan extraction with typical peaks at 3348.78, 2919.70, and 1635.34 cm⁻¹. Chitosan displayed concentration-dependent antioxidant activity with DPPH radical scavenging of $18.18 \pm 1.29\%$ to $90.91 \pm 1.29\%$ (100–1000 $\mu\text{g/mL}$). The results showed antibacterial activity with inhibition zones of 22.0 ± 0.6 mm against *Staphylococcusaureus* and 21.0 ± 0.7 mm against *Escherichia coli*. The biocomposite covering enhanced shelf life of grapes from 5-12 days, bananas from 6-14 days, tomatoes from 10-18 days and cucumbers from 7-13 days by cutting moisture loss, delaying ripening, retaining firmness and minimising microbiological deterioration. **Novelty:** The research provides a multifunctional biodegradable chitosan–cellulose–green tea extract coating with antibacterial, antioxidant, and barrier capabilities. Unlike previous studies that have been mainly conducted on chitosan alone or on a single fruit commodity, the present study couples microbial profiling with the effective use of a composite coating on multiple fruits and vegetables, showing sustainable alternative to synthetic preservatives and packaging materials.

Keywords: Chitosan; Microbial diversity; Cellulose; Green tea extract; Postharvest; preservation

1 Introduction

Chitosan, a deacetylated derivative of chitin, has attracted tremendous attention for its application in food preservation because of its biodegradability, biocompatibility, film-forming ability, and natural antibacterial and antioxidant capabilities⁽¹⁾. Chitosan coatings are semi-permeable barriers that regulate gas exchange and moisture transfer and reduce microbial decomposition, thereby extending the shelf life of fruits and vegetables⁽²⁾. The use of cellulose from agro-industrial wastes such as coir pith has been explored as a reinforcing ingredient in chitosan-based coatings to enhance their mechanical strength, stability, and water resistance⁽³⁾. Green tea extract, being a rich source of polyphenolic compounds with significant antioxidant and antibacterial activity, can increase the preservation efficacy of edible coatings⁽⁴⁾. However, great breakthroughs have been achieved, although some limits still exist in the field of chitosan-based preservation methods. Most previous work has focused on chitosan coatings, physicochemical characterisation of coating ingredients, or applications to a single fruit or vegetable⁽⁵⁾. In addition, conventional chitosan coatings are poor in mechanical strength, moisture barrier, and antioxidant activity, which may influence their long-term preservation performance. Moreover, there is a lack of studies on the relationship between spoilage-associated microbial communities and the effectiveness of biodegradable composite covers for reducing postharvest degradation. Therefore, multifunctional sustainable coating technologies are needed to simultaneously address microbiological decomposition, moisture loss, softness, discoloration, and reduced shelf life in fresh fruit.

In the present work, these limitations were overcome by developing a chitosan-cellulose-green tea bio composite coating. Chitosan was used because of its antibacterial and film-forming properties, cellulose was isolated from coir pith to improve the structural integrity and moisture resistance, and green tea extract was added to give additional antioxidant and antimicrobial activity. The suggested technique overcomes the main postharvest difficulties such as microbial contamination, quality loss, quick ripening and short shelf life of fruits and vegetables. The uniqueness of this study is to combine the microbial diversity profiles of naturally damaged tomato, cucumber, banana and grape samples with the development and testing of a multifunctional biodegradable coating system. Unlike previous studies that solely investigated preservation performance, the present study directly related spoilage-associated bacteria to the efficacy of a sustainable preservation strategy. Moreover, the utilisation of chitosan from shrimp shell and cellulose from coir pith promote waste valorisation and environmental sustainability. This work describes a novel chitosan-cellulose-green tea biocomposite coating for postharvest fruit preservation via microbial characterisation, antioxidant and antibacterial evaluation and shelf-life extension using an eco-friendly and holistic approach.

2 Methodology

2.1 Chitosan Extraction

The shrimp shells were collected from a local fish market at Suler, Coimbatore, Tamil Nadu for the extraction of chitin and chitosan. Chitosan was prepared from prawn shells by deproteinization in alkaline medium, demineralization in acid medium, deacetylation and decolorization⁽⁶⁾.

2.1.1. Deproteinization

Alkaline methods were employed to remove protein from prawn shells. Prawn shells were treated with 4% NaOH at 80 °C for 8 h. The solution was filtered and washed several times with distilled water until the pH was near neutral, suggesting protein removal⁽⁶⁾.

2.1.2. De-mineralisation

Shrimp shells were treated with 4 % HCl at 37 °C for 12 h. After acid treatment, samples were rinsed with distilled water until a neutral pH was reached⁽⁷⁾.

2.1.3. Deacetylation

Chitin was suspended in 50% (w/v) NaOH and heated at 100 °C for 6 h to hydrolyze the acetamido links. Purified Chitosan powder⁽⁸⁾ was obtained by washing the reaction mixtures with distilled water, centrifuging to neutral pH, and oven drying at 40 °C for 12 h.

2.1.4. Decolorisation

The isolated chitin was bleached with 1% potassium permanganate for 1 h and 1% oxalic acid for 30 min. The purified white chitosan was washed with distilled water until the pH became neutral and then dried at 60 °C for 24 h⁽⁹⁾.

2.2 Characterisation of Chitosan from Shrimp

2.2.1. Fourier transform infrared (FTIR) spectroscopy

FTIR analysis was performed on a PerkinElmer Spectrum 100 spectrophotometer equipped with a Universal Attenuated Total Reflectance (ATR) attachment. 1 mg dried chitosan powder was coated on the ATR crystal and the spectra were recorded at a resolution of 4 cm⁻² in the range of 4000-400 cm⁻¹. To confirm the chemical structure of the chitosan, about 1mg of chitosan was subjected to test for typical functional groups including O-H, N-H and amide bands⁽¹⁰⁾.

2.3 Study of Microbes in Rotten Fruits and Vegetables.

Spoiled tomato (*Solanum lycopersicum*), cucumber (*Cucumis sativus*), banana (*Musa paradisiaca*) and grape (*Vitis vinifera*) exhibiting clear signs of spoilage such as discolouration, softening, tissue deterioration and fungal growth were collected from Sular Market, Coimbatore, Tamil Nadu, India. The samples were collected in aseptic containers and transported to the laboratory for microbiological analysis. Isolation of bacteria was done on selective and differential media by serial dilution and spread plate technique. The cultured bacterial colonies were then purified and subjected to analysis based on colony morphology, pigmentation, consistency, Gram's staining reaction, and biochemical tests⁽¹¹⁾. All experiments were performed in pairs.

2.4 DPPH Test for Antioxidant Activity

The antioxidant activity of chitosan was determined by DPPH free radical scavenging assay. A freshly prepared 0.1 mM DPPH solution was incubated with chitosan solutions (100–1000 µg/mL) in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using UV-Visible spectrophotometer. DPPH solution without chitosan was served as control. All measurements were carried out in duplicate. The antioxidant activity was represented as % DPPH radical scavenging⁽¹²⁾.

$$\text{Scavenging activity (\%)} = [(A_{517} \text{ of control} - A_{517} \text{ of sample}) / (A_{517} \text{ of control})] \times 100$$

2.5 Antibacterial Activity

The antibacterial activity of chitosan was tested against bacterial isolates from spoiled tomato, cucumber, grape and banana using agar well diffusion method. Bacterial suspensions were diluted to approximately 1×10^6 CFU/mL (0.5 McFarland standard) and inoculation onto Mueller-Hinton agar plates. Wells of 6 mm diameter were punched in agar and filled with chitosan solutions (500, 750 and 1000 µg/mL). The positive control was streptomycin (10 µg/mL). The plates were incubated for 24 h at 37 °C and the zones of inhibition were measured in mm. All experiments were performed in duplicate⁽¹³⁾.

2.6 Cellulose Extraction from Coir Pith

Coir pith was washed, dried, alkali-treated with NaOH to remove lignin and hemicellulose, hydrolyzed with HCl, washed well, dried, and powdered⁽¹⁴⁾.

2.7 Preparation of Green Tea Extract (GTE)

Green tea powder was extracted with hot distilled water (1:5, w/v) at 90 °C for 10 min, filtered and the filtrate used for composite preparation⁽¹⁴⁾

2.8 Formulation of Composite Chitosan Coating

An edible coating solution was prepared by mixing 1% (w/v) chitosan solution in 1% acetic acid with 0.5% coir pith cellulose and 0.5% green tea extract, and homogenised and degassed.

2.9 Application of Composite Coatings

Fresh tomato (*Solanum lycopersicum*), banana (*Musa paradisiaca*), cucumber (*Cucumis sativus*), and grapes (*Vitis vinifera*) were collected from Sular Market, Coimbatore, Tamil Nadu, India during the winter season (December 2025). Fruits and vegetables of the same size, maturity, and free from any evident physical damage or signs of illness were chosen for the study. Each commodity was used in twenty samples and randomly divided into coated and uncoated (control) groups. Each treatment was independently replicated three times.

2.9.1. Preparation of Chitosan-Based Biocomposite and Coatings on fruits and vegetables

The coating solution was produced by dissolving chitosan in 1% (v/v) acetic acid under constant stirring. The chitosan solution was supplemented with cellulose derived from coir pith and green tea extract, and plasticizer glycerol was added. The mixture was homogenized to a homogeneous coating solution. A completely randomized design (CRD) was used with two treatments: (i) coated samples and (ii) uncoated control samples. Fruits and vegetables assigned to the coated treatment were dipped in the coating solution for 2 min, then drained and air-dried at room temperature. All samples were kept in the same ambient conditions ($27 \pm 2^\circ\text{C}$; relative humidity 65–75%) and monitored⁽¹⁵⁾.

2.9.2. Assessment of Shelf-Life Extension in Coated Fruits and Vegetables

The shelf-life of coated and uncoated tomato, banana, cucumber and grape samples were studied at ambient storage conditions ($27 \pm 2^\circ\text{C}$ and 65–75% relative humidity). Samples were monitored on days 0, 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19 of storage. The assessment was done on both quantitative and qualitative parameters for the course of postharvest degradation and spoilage⁽¹⁵⁾. Quantitative assessment was done by weighing the individual samples at the beginning of the storage and at each observation interval using a weighing balance. We computed the percentage of weight loss during the storage using the following equation:

$$\text{Weight loss (\%)} = \frac{\text{Initial weight} - \text{Weight at observation day}}{\text{Initial weight}} \times 100$$

The difference of weight was taken as a quantitative parameter of moisture loss and deterioration during storage. To give a qualitative spoiling assessment, the exterior quality of the samples was examined at each observation interval. The factors tested were colour retention, firmness/stiffness, shrivelling, softening, tissue degeneration, obvious microbial development on the surface and overall marketability. Coated and uncoated control samples were evaluated by the same criteria of assessment during the whole storage period. Samples that retained good colour and appearance, hardness, absence of noticeable shrivelling or softening and no obvious microbiological growth were regarded to be of acceptable quality. Progressive deterioration in colour, loss of firmness, shrivelling, softening, degradation of tissue and apparent microbial proliferation indicated growing spoiling. Samples with substantial degradation and loss of marketability were determined to have attained the spoiling endpoint. Shelf-life was defined as the number of days the samples were acceptable and marketable before the spoiling endpoint⁽¹⁵⁾.

2.10 Statistical Analysis

All experimental data are presented as the mean $\pm$ standard error (SE) of triplicate measurements. Statistical analysis of data on antibacterial activity, antioxidant activity, and preservation efficiency of chitosan-based composite coatings was done by one-way analysis of variance (ANOVA) using SPSS software (version 16.0). Duncan's Multiple Range Test (DMRT) at a 95% confidence level was used to assess significant differences across treatment means. A p-value of <0.05 was interpreted as statistically significant.

3 Result and Discussion

3.1 Extraction of chitosan from shrimp shell

The extracted shrimp shell chitosan (SSCS) was a pure white powder demonstrating the efficient removal of proteins, minerals and colours during the extraction process as shown in Figure 1.



Fig 1. Chitosan from shrimp shell

3.2 Structural and spectroscopic characterisation of SSCS

3.2.1. Fourier Transform Infrared (FTIR) Spectrum

FTIR spectra confirmed the presence of typical functional groups of chitosan. The broad absorption band at 348.78 cm^{-1} was assigned to the O-H and N-H stretching vibrations, and the peak at 2919.7 cm^{-1} is due to the C-H stretching vibrations. The amide I band at 1635.34 cm^{-1} is assigned to C=O stretching, and the peak at 1377.89 cm^{-1} is assigned to CH_3 bending. The peaks at 1149.37 and 1027.87 cm^{-1} were assigned to C-O-C and C-O vibrations of the polysaccharide backbone. The results indicated the effective extraction of chitosan and agreed with prior reports⁽¹⁶⁾ (Figure 2).

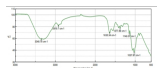


Fig 2. Fourier transform infrared spectrum of extracted chitosan

3.3 Microbial profiling from spoiled fruits (grapes and banana) and vegetables (tomatoes and cucumbers)

3.3.1. Profiling and authentication of bacterial isolates from spoiled tomato

Distinct colonies that developed after incubation of serially diluted spoiled tomato samples collected from Sullur, Coimbatore, Tamil Nadu, India, during the winter season (December 2025). The microorganisms isolated from the spoiled tomato samples were identified based on cultural, morphological, microscopic, and biochemical properties⁽¹¹⁾. Thirteen bacterial isolates were obtained on selective media including *Escherichia coli*, *Klebsiella* spp, *Shigella* spp, *Pseudomonas* spp, *Bacillus* spp and *Staphylococcus* spp. Gram staining and biochemical assays (IMViC, TSI, urease, catalase and oxidase) validated the identification (Table 1). *Escherichia coli*, *Klebsiella* spp. and *Shigella* spp. indicators of potential contamination via soil, irrigation water or unclean handling during production and post-harvest processing, all of which point to possible food safety problems⁽¹⁷⁾.

Table 1. Identification of Bacteria from Spoiled Tomato Samples

S. No.	Isolated Bacteria	Gram Staining	Indole (I)	MR	VP	Citrate (C)	TSI	Urease (U)	Catalase	Oxidase	Identified Organism
1	Sample 1	–	–	+	+	+	A/A, gas	+	+	–	<i>Klebsiella</i> sp.
2	Sample 2	–	–	–	–	+	K/K	–	+	+	<i>Pseudomonas</i> sp.
3	Sample 3	+	–	+	+	+	A/A	–	+	+	<i>Bacillus</i> sp.
4	Sample 4	+	–	+	+	–	A/A	+	+	–	<i>Staphylococcus</i> sp.
5	Sample 5	–	–	–	–	+	K/K	–	+	+	<i>Pseudomonas</i> sp.
6	Sample 6	+	–	+	+	+	A/A	–	+	+	<i>Bacillus</i> sp.
7	Sample 7	–	+	+	–	–	A/A, gas	–	+	–	<i>E. coli</i>
8	Sample 8	–	–	+	+	+	A/A, gas	+	+	–	<i>Klebsiella</i> sp.
9	Sample 9	+	–	+	+	–	A/A	+	+	–	<i>Staphylococcus</i> sp.
10	Sample 10	–	+	+	–	–	K/A	–	+	–	<i>Shigella</i> sp.
11	Sample 11	–	+	+	–	–	A/A, gas	–	+	–	<i>E. coli</i>
12	Sample 12	+	–	+	+	+	A/A	–	+	+	<i>Bacillus</i> sp.
13	Sample 13	+	–	+	+	+	A/A	–	+	+	<i>Bacillus</i> sp.

3.3.2. Profiling and authentication of bacterial isolates from spoiled cucumber

Serially diluted spoiled cucumber samples showed distinct colonies. The predominant isolate was *Bacillus* spp. (25%) followed by *Escherichia coli*, *Enterobacter* spp., *Pseudomonas* spp. and *Lactobacillus* spp. (16.7%) while *Salmonella* spp. accounted for

8.3% of the isolates (Table 2). *Bacillus* and *Pseudomonas* were identified as implicated in the rotting of cucumber, whereas *Lactobacillus* was involved in natural fermentation. The presence of *E. coli* and *Salmonella* may indicate contamination from soil, irrigation water, or handling. The results are in agreement with recent studies on cucumber spoilage bacteria⁽¹⁸⁾.

Table 2. Identification of Bacteria Isolated from Spoiled Cucumber Samples.

S. No	Isolated Bacteria	Gram Staining	Indole	MR	VP	Cit-rate	TSI	Ure-ase	Cata-lase	Oxi-dase	Identified Organism
1	Sample 1	–	+	+	–	–	A/A gas	–	+	–	<i>E. coli</i>
2	Sample 2	–	–	+	–	+	K/A	–	+	–	<i>Enterobacter</i>
3	Sample 3	+	–	+	–	–	A/A	–	+	–	<i>Bacillus</i>
4	Sample 4	+	–	+	–	–	A/A	–	+	–	<i>Bacillus</i>
5	Sample 5	–	+	+	–	–	A/A gas	–	+	–	<i>E. coli</i>
6	Sample 6	–	–	+	–	+	K/A	–	+	+	<i>Pseudomonas</i>
7	Sample 7	+	–	–	+	+	A/A	+	+	–	<i>Lactobacillus</i>
8	Sample 8	+	–	+	–	–	A/A	–	+	–	<i>Bacillus</i>
9	Sample 9	–	–	+	–	+	K/A	–	+	+	<i>Pseudomonas</i>
10	Sample 10	+	–	–	+	+	A/A	+	+	–	<i>Lactobacillus</i>
11	Sample 11	–	–	+	–	–	K/A	–	+	–	<i>Salmonella</i>
12	Sample 12	–	–	+	+	+	A/A gas	+	+	–	<i>Enterobacter</i>

3.3.3. Profiling and authentication of bacterial isolates from spoiled Banana

The serial dilution of spoiled banana samples revealed significant microbial growth linked with fruit deterioration, leading to distinct bacterial colonies. Biochemical and Gram staining analysis showed *Staphylococcus* spp., *Bacillus* spp., *Enterococcus* spp., *Klebsiella* spp., *Enterobacter* spp., and *Vibrio* spp. (Table 3). *Staphylococcus* spp. (27.3%) followed by *Bacillus* spp. and *Enterococcus* spp. as the commonest isolates. (18.2% each). The role of these bacteria in enzymatic degradation and fermentation activities that contribute to banana deterioration, and the presence of *Klebsiella* and *Enterobacter*, indicate contamination and faster rotting processes⁽¹⁹⁾.

Table 3. Identification of Bacteria Isolated from Banana Samples.

S. No	Isolated Bacteria	Gram Staining	Indole	MR	VP	Cit-rate	TSI	Ure-ase	Cata-lase	Oxi-dase	Identified Organism
1	Sample 1	+	–	+	–	–	A/A	–	+	–	<i>Bacillus</i>
2	Sample 2	+	–	+	+	+	A/A	–	+	–	<i>Staphylococcus</i>
3	Sample 3	–	–	+	–	–	A/A	–	+	–	<i>Enterococcus</i>
4	Sample 4	–	–	+	+	+	A/A gas	+	+	–	<i>Klebsiella</i>
5	Sample 5	+	–	+	+	–	A/A	–	+	–	<i>Staphylococcus</i>
6	Sample 6	–	–	+	–	–	K/A	–	+	–	<i>Enterobacter</i>
7	Sample 7	+	–	+	+	+	A/A	–	+	–	<i>Staphylococcus</i>
8	Sample 8	+	–	+	–	–	A/A	–	+	–	<i>Bacillus</i>
9	Sample 9	–	+	+	–	+	K/A	–	+	+	<i>Vibrio</i>
10	Sample 10	–	–	+	+	+	A/A gas	+	+	–	<i>Klebsiella</i>
11	Sample 11	–	–	+	–	–	A/A	–	+	–	<i>Enterococcus</i>

3.3.4. Profiling and authentication of bacterial isolates from spoiled grapes.

Spoiled grapes revealed abundant bacterial growth, and the colonies showed various morphologies, indicating the presence of multiple bacterial species. Thirteen isolates were identified as *Bacillus* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Enterococcus* spp. and *Klebsiella* spp. (Table 4). The most abundant strain was *Bacillus* spp. (30.8%) followed by *Staphylococcus* spp. and *Pseudomonas* spp. (23.1% each). These bacteria are known to cause deterioration of fruits by enzymatic degradation, fermentation, and loss of quality. Environmental or handling contamination may lead to the presence of *Staphylococcus* and *Klebsiella*. The diversity of isolated microbes is consistent with prior studies of grape rotting bacteria.⁽¹⁹⁾

Table 4. Identification of Bacteria Isolated from Grape Samples.

S. No	Isolated Bacteria	Gram Staining	Indole	MR	VP	Cit-rate	TSI	Ure-ase	Cata-lase	Oxi-dase	Identified Organism
1	Sample 1	–	–	+	–	+	K/K	–	+	+	<i>Pseudomonas</i>
2	Sample 2	+	–	+	–	–	A/A	–	+	–	<i>Bacillus</i>
3	Sample 3	–	–	+	+	+	A/A gas	+	+	–	<i>Klebsiella</i>
4	Sample 4	+	–	+	–	–	A/A	–	+	–	<i>Enterococcus</i>
5	Sample 5	+	–	+	–	–	A/A	–	+	–	<i>Bacillus</i>
6	Sample 6	+	–	+	+	+	A/A	+	+	–	<i>Staphylococcus</i>
7	Sample 7	–	–	+	–	+	K/K	–	+	+	<i>Pseudomonas</i>
8	Sample 8	+	–	+	–	–	A/A	–	+	–	<i>Enterococcus</i>
9	Sample 9	+	–	+	–	–	A/A	–	+	–	<i>Bacillus</i>
10	Sample 10	+	–	+	+	+	A/A	+	+	–	<i>Staphylococcus</i>
11	Sample 11	+	–	+	–	–	A/A	–	+	–	<i>Bacillus</i>
12	Sample 12	–	–	+	–	+	K/K	–	+	+	<i>Pseudomonas</i>
13	Sample 13	+	–	+	+	+	A/A	+	+	–	<i>Staphylococcus</i>

3.4 Antioxidant activity of the extracted chitosan by using DPPH assay

The antioxidant activity of the isolated chitosan increased in a concentration-dependent manner from $18.18 \pm 1.29\%$ at $100 \mu\text{g/mL}$ to $90.91 \pm 1.29\%$ at $1000 \mu\text{g/mL}$ for DPPH scavenging activity. Similar antioxidant capabilities of chitosan were also reported in recent investigations under similar doses, with scavenging activities ranging from 70–88%. The minor increase in antioxidant activity in the present study may be related to the high degree of deacetylation and purity of the isolated shrimp-shell chitosan⁽²⁰⁾. This strong antioxidant capacity is especially significant in postharvest conservation, because it helps prevent oxidative damage, delay pigment degradation and maintain quality features during storage. The results indicate the use of chitosan as an active component of edible coating formulations.

The extracted chitosan showed a broad spectrum antibacterial activity against Gram positive and Gram negative bacteria with maximum inhibition of 22.0 ± 0.6 mm against *Staphylococcus aureus*. Similar antibacterial activity of chitosan has been observed in subsequent investigations, with inhibition zones generally between 15–20 mm against food spoilage bacteria, indicating its usefulness as a natural antimicrobial agent⁽²¹⁾. The relatively bigger inhibitory zones reported in this study may be related to the molecular properties of chitosan from prawn shells and its strong interaction with the bacterial cell membranes. The antibacterial activity of chitosan is due mainly to electrostatic contact between the positively charged amino groups of the molecule and the negatively charged bacterial cell surface, resulting in rupture of the membrane and leakage of internal components.

3.5 Identification of antibacterial activity of chitosan

Chitosan exhibited broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria, and activity increased with increasing concentration. The least sensitive bacteria were *Pseudomonas aeruginosa* and the most sensitive was *Staphylococcus aureus*. (Figure 3, Table 5). The antibacterial activity detected was similar to or higher than that reported in recent studies of chitosan-based antimicrobial materials, showing the efficacy of chitosan derived from prawn shells against food spoilage bacteria⁽²²⁾.

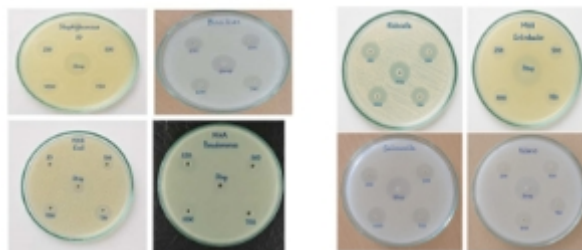


Fig 3. Antibacterial activity of chitosan

3.6 Antioxidant activity of the extracted chitosan by using DPPH assay

The antibacterial activity is due to the electrostatic interaction between the positively charged molecules of chitosan and the negatively charged surfaces of the bacterial cells. This causes the cell to die and the membrane to collapse. Also, the incorporation of cellulose and green tea extract increased the coating performance by improving its barrier, antioxidant and antibacterial activities. The developed chitosan-cellulose-green tea biocomposite was effective in preserving multiple fruits and vegetables unlike many previous studies on single component coatings or one commodity, which is a novelty, and its potential for sustainable postharvest preservation is highlighted.

Table 5. Identification of antibacterial activity of chitosan

Bacterial pathogens	Concentration ($\mu\text{g/mL}$) / Zone of inhibition (mm)				Std Drug (S 30 μg)
	250	500	750	1000	
Gram-positive bacteria					
<i>B. subtilis</i>	11.0 $\pm$ 0.5	14.5 $\pm$ 0.6	18.0 $\pm$ 0.5	20.0 $\pm$ 0.6	25.0 $\pm$ 0.5
<i>S. aureus</i>	9.5 $\pm$ 0.4	14.0 $\pm$ 0.5	19.0 $\pm$ 0.5	22.0 $\pm$ 0.6	25.6 $\pm$ 0.4
Gram-negative bacteria					
<i>E. coli</i>	8.5 $\pm$ 0.5	13 $\pm$ 0.6	15.0 $\pm$ 0.5	21.0 $\pm$ 0.7	27.0 $\pm$ 0.10
<i>P. aeruginosa</i>	7.5 $\pm$ 0.5	9.0 $\pm$ 0.5	13.0 $\pm$ 0.6	16.3 $\pm$ 0.5	21.3 $\pm$ 0.5
<i>K. pnumoniae</i>	7.6 $\pm$ 0.4	12.0 $\pm$ 0.5	14.6 $\pm$ 0.6	20.0 $\pm$ 0.5	23.0 $\pm$ 0.5
<i>Enterobacter sp.</i>	9.0 $\pm$ 0.5	12.5 $\pm$ 0.4	15.0 $\pm$ 0.5	18.0 $\pm$ 0.6	21.0 $\pm$ 0.4
<i>Salmonella sp.</i>	8.5 $\pm$ 0.4	11.8 $\pm$ 0.5	15.0 $\pm$ 0.4	21.0 $\pm$ 0.5	24.0 $\pm$ 0.4
<i>Vibrio sp</i>	9.8 $\pm$ 0.4	13.0 $\pm$ 0.6	17.5 $\pm$ 0.5	20.0 $\pm$ 0.6	23.0 $\pm$ 0.4

3.7 Impact of Chitosan Composite Coating on Shelf Life

The chitosan-cellulose-green tea composite coating was applied to grapes, bananas, tomatoes and cucumbers by the dipping method (Figure 4). Shelf-life was determined by quantitative weight loss and qualitative changes in colour, hardness, shrivelling, softening, visible microbiological growth and marketability on days 0, 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19. The present approach is in agreement with the current practice for the assessment of shelf-life of fruits and vegetables. The coated samples often exhibited better colour retention, hardness and overall appearance, with delayed shrivelling, softening and apparent microbiological degradation than the uncoated controls. Weight loss was lower in coated tomatoes (3.70%) and grapes (1.80%) than in their controls (10.09% and 2.52%, respectively). For banana and cucumber, weight reduction was 9.93 and 20.41% in coated samples compared with 9.14 and 18.33% in controls, respectively, demonstrating a commodity-dependent effect.

Total quality assessment of the coated samples revealed that the samples were marketable for 12 days for grapes, 14 days for bananas, 18 days for tomatoes and 13 days for cucumbers as against the uncoated controls of 5, 6, 10 and 7 days, respectively. Thus, the marketable storage duration was increased by around 7, 8, 8 and 6 days, respectively. The better quality retention could be due to the combination film forming, barrier and bioactive capabilities of chitosan, cellulose and green tea extract. In general, the composite covering delayed the postharvest deterioration and increased the storage quality of the studied fruits and vegetables⁽²³⁾.

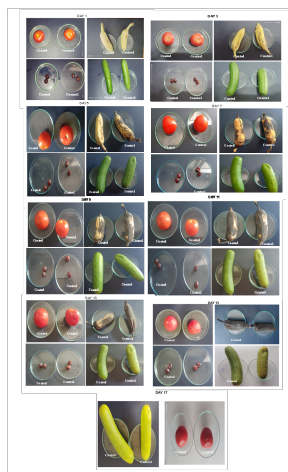


Fig 4. Chitosan composite-coated and uncoated (control) for tomato, banana, grapes, and cucumber

4 Conclusion

The study successfully addressed the aims of studying the microbial diversity of damaged fruits and vegetables and developing a biodegradable chitosan-cellulose-green tea extract biocomposite layer for postharvest preservation. Microbial profiling of spoiled samples of tomato, cucumber, banana and grape showed diverse spoilage-associated bacteria, including *Escherichia coli*, *Bacillus* spp, *Staphylococcus* spp, *Pseudomonas* spp, *Klebsiella* spp and *Enterobacter* spp. This emphasizes the great importance of microbial contamination in postharvest spoilage. The isolated shrimp-shell chitosan was characterised successfully by FTIR study indicating its structural integrity and potential for coating applications. The antioxidant activity of chitosan was pronounced and increased from 18.18 ± 1.29 % to 90.91 ± 1.29 % with the increase in concentration from 100 $\mu\text{g/mL}$ to 1000 $\mu\text{g/mL}$. Broad range antibacterial activity was shown by chitosan with highest zone of inhibition seen against *Staphylococcus aureus* (22.0 ± 0.6 mm). The new application of the chitosan–cellulose–green tea extract coating has significant impact on the storage stability of fresh food. The shelf life was extended by 5-12 days in grapes, 6-14 days in bananas, 10-18 days in tomatoes and 7-13 days in cucumbers as compared to uncoated controls. The coating effectively controlled moisture loss, retarded ripening and softening, sustained firmness and minimised microbial damage during storage. Our approach is unusual since it uses chitosan, cellulose and green tea extract in a multifunctional coating system. It combines antibacterial, antioxidant and barrier qualities in one biodegradable molecule. However, in the present investigation, the composite covering was shown to be effective for numerous fruits and vegetables. Previous research have often concentrated on chitosan alone or on a single fruit product. This indicates broader application for postharvest preservation. The results indicate that the created biocomposite coating is a simple, low cost, eco- friendly and sustainable alternative to the existing chemical preservatives and synthetic packaging materials. Future research should be aimed at optimising the coating formulation, assessing the physicochemical and sensory quality parameters during long-term storage, investigating large-scale commercial applications, and studying the effectiveness of the coating under different storage conditions. Further research on other horticultural commodities and more advanced characterisation techniques will lead to the implementation and monetisation of this potential postharvest preservation solution.

5 Acknowledgements

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Author contributions: Kiruthiga Periyannan: Conceptualisation, formal analysis, writing—original draft; Balachandar Subbu: Supervision; Hemamala Selvaraj: Visualisation.

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