

# Biopolymer-Based Natural Antimicrobial Coatings for Food-Contact Surfaces and Their Role in Food Safety and Microbial Diversity



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## ABSTRACT

Preventing contamination with pathogenic and spoilage microbes is very important to keep surfaces clean in the environment of food and beverage production. Traditional chemical sanitizers have limitations including residues, corrosion of equipment, user safety and possible microorganism adaptations. In the present investigation, naturally based (biopolymers) antimicrobial coatings were prepared by combining sodium alginate with five natural agents of clove, garlic, turmeric, honey and vinegar (Acetic Acid), tested on glass slides as surrogate food contact surfaces. The coatings were evaluated against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Serratia marcescens*, and mixed bacterial cultures using optical density and viable plate count methods. CFU/cm<sup>2</sup>, log<sub>10</sub> reduction, and percentage reduction were determined, and coatings were ranked based on antimicrobial efficacy, broad-spectrum activity, statistical significance, and practical applicability. Among all tested formulations, clove-alginate exhibited the highest antibacterial activity, achieving >2 log<sub>10</sub> and >97% reduction against both Gram-positive and Gram-negative bacteria, followed by turmeric and acetic acid-alginate coatings with moderate efficacy. Honey and garlic alginate coatings demonstrated limited or inconsistent antimicrobial effects. These findings suggest that clove-alginate acts as a safe, effective and long-lasting substitute to commercial chemical sanitizers, representing an environmentally friendly alternative for surface protection in contact with food. This work demonstrates the potential of natural extract-based biopolymer coatings as a viable means of improving hygiene and safety in food systems.

**Keywords:** Natural antimicrobial coatings, Sodium alginate, Clove extract, Food-contact surfaces, Biopolymer-based hygiene, Sustainable food safety.

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## INTRODUCTION

Maintaining a safe microbial environment for food-contact surfaces is a major requirement in the practice of food canning, maintaining goods properly, and distribution of goods. Once contaminated, these surfaces become constant reservoirs of pathogenic (disease-causing) germs, as well as decay microorganisms [1]. This contamination produces a wide spectrum of microbes which will eventually find their way onto products, in many cases making them unfit to eat, in others they simply shorten their shelf life. This symbolic threat to the preservation and enhancement of foodstuffs' taste, which has been our lifeline through so much hardship past generations experienced, is expressed by its common use today at every level. Pathogenic organisms like *Staphylococcus aureus*, *Serratia marcescens* and *Pseudomonas aeruginosa* pose a great health hazard [2]. They can survive on the surfaces of commonly used materials, such as stainless steel, plastic, and glass even after these have been cleaned in compliance with regulations. It is indeed difficult to remove them. They can form inconspicuous biofilms on clean surfaces, cling tightly to lifeless inorganic matter, survive without water, and render useless or unable to

kill some of our most effective sanitizers [3]. These resistant factors lead to the recurrence of contamination incidents, drawing attention back time and again to effective sanitation strategies as well as the need for better understanding about how pathogens remain on contact surfaces for long periods of time and in hard-to-reach spots [4].

Hygiene barrier procedures in the entire food industry sector include the use of chemical sanitizers, including compounds based on chloride, quaternary ammonium salts, hydrogen peroxide, and alcohols. In general, such chemicals are capable of reducing the microbial count provided that they are correctly applied [5]. But the frequent and repeated use of them has made several drawbacks, which seriously influence food safety measures as well as overall operation efficiency. A lot of sanitizers can leave undesirable chemical deposits that may make food products unsafe for consumption or may need added rinsing to comply with health regulations [6]. Some of these agents are corrosive and or have deleterious effects on processing equipment, with stainless steel surfaces, plastic parts gaskets and seals suffering long-term damage that will increase maintenance costs. Beyond equipment issues,

employees who come into contact with high concentrations of chemical sanitizers can also suffer from skin irritation, breathing difficulties and other occupational health hazards [7]. A related concern for this latter reason is the possible pathogen resistance or tolerance which may develop following gradual exposure to sublethal doses of sanitizers. Consistently, consumer demand is moving toward clean-label foods and environmentally responsible food processing practices [8]. This has driven the demand for environment-friendly, chemical-free and natural sanitation practices. Consequently, attention in scientific research is trending towards alternative approaches like green natural biodegradable food safe antimicrobial coatings [9]. By contrast to traditional professional-use disinfectants, these coatings may also provide the possibility of long-term antimicrobial protection through cycled cleaning efforts, thus further reducing the risk for melt-scum persistence and surface recontamination. Their creation is part of the process to provide sustainable, efficient and user-friendly hygiene solutions in food production.

Biopolymer coatings have received great interest as a new and sustainable method for replacing traditional sanitization means, mainly because biopolymeric coatings can form stable protecting film providing encapsulation of active ingredients and the release of antimicrobial agents in a controlled and sustained manner [10]. These sustainable materials offer an environmentally responsible substrate for creating surfaces that are able to limit microorganism growth over periods of time between cleaning. Sodium alginate is one of the most commonly used and researched biopolymers among other investigated materials. Due to its biocompatible, biodegradable, non-toxic and Generally Recognized as Safe (GRAS) status it is very suitable for food and bio-medical applications [11]. Sodium alginate also has properties of forming good films, which can form in accordance with a substrates to be coated and exhibits excellent characteristics such as flexible properties. Due to this fact it has been widely used in food preservation systems, edible films, wound dressings as well as pharmaceutical delivery and toxicity studies that confirm its diverse range and toxicological safety [12]. One of the most attractive properties of alginate is that, being able to undergo ion-induced gelation, it can be used to efficiently entrap several different natural antimicrobial agents (such as essential oils, plant extracts, organic acids and nanoparticles) [13]. Such gel forming ability enables a controlled long term release of bioactive agents as deposited onto soiled surfaces with the result of strengthening its antimicrobial efficacy and extending the duration of protection.

Naturally-occurring antimicrobials, including clove extract (eugenol), garlic extract (allicin), turmeric extract (curcuminoids), vinegar (acetic acid) and honey (phenolics, hydrogen peroxide; very significant osmotic effect) have shown promising antibacterial action against a wide variety of bacteria. These bioactive compounds act by several mechanisms, such as permeabilization of the cell membranes, denaturation of essential proteins, inhibition and disruption of metabolic enzymes in cells, interfering with respiration pathways in the cell, chelating important metal ions to living organism and induction of oxidative stress [14]. Their multi-target modes of action have resulted in application and effectiveness against a broad spectrum of foodborne pathogens and spoilage organisms. Besides being bactericidal, several practical benefits can be derived from these natural compounds.

They are low-cost, highly available compounds that are environmentally friendly and found safe for direct or indirect contact with food, which coincides with consumer preferences towards natural and chemical-free preservation methods. They have been widely used for centuries in traditional medicine and food preservation; therefore long-term safety and effectiveness are supported [15].

Despite their good properties, the utilization of these latter natural antimicrobials as included surface protective coatings, as a component into or blended with biopolymers has not been deeply investigated up to now. Their capacity to create stable and long-lasting AM layers on food-contact surfaces is not well documented in the literature, nor has it been fully explored through systematic scientific studies or compared among them, which clearly indicates a missing link addressing their potential application within modern food safety systems [16]. While all-natural antimicrobials have been tested in food preservation and edible film, they have remained relatively unused as coatings of surfaces coming into direct contact with food. Critical gaps are: no comparison studies of several natural agents, limited testing with mixed (Gram-positive and Gram-negative) bacterial models, and inadequate use of both optical density and plate count for quantification. Furthermore, durability, wiping resistance and practical industrial concerns of implementing such surfaces (feasibility, cost and safety) are hardly ever studied. Such gaps would argue for a more systematic evaluation of the antimicrobial properties of natural coatings that can be used regularly in food processing facilities [17].

In the current study, five natural antimicrobial compounds including clove, garlic, turmeric, vinegar and honey were combined with sodium alginate to develop biopolymer-based antimicrobial coatings. Glass slides served as a model food-contact surface. The coatings were challenged with *Staphylococcus aureus*, *Serratia marcescens*, and *Pseudomonas aeruginosa* from both single and mixed inocula. Antimicrobial activity was assessed by optical density and viable plate counts, while durability, wipe resistance, and performance during storage were measured in comparison to that of uncoated slides. This study aims to identify a safe, affordable, and effective natural antimicrobial coating that can serve as an alternative to conventional chemical sanitizers. By demonstrating strong antimicrobial performance and practical durability, the findings are expected to advance sustainable hygiene practices in the food industry and encourage the adoption of eco-friendly, biopolymer-based antimicrobial technologies for routine surface protection.

## MATERIALS AND METHODOLOGY

**Preparation of Alginate-Based Natural Antimicrobial Coatings-** Alginate-based antimicrobial coatings were prepared using sodium alginate (2% w/v) as the biopolymer matrix. A 2% alginate stock solution was produced by dissolving 2.0 g sodium alginate in warm distilled water (40–45°C) with continuous stirring until a uniform gel-like solution was obtained and adjusting the final volume to 100 mL. Five natural antimicrobial agents, clove oil, garlic extract, turmeric powder, honey, and vinegar were incorporated individually into 10 mL aliquots of the alginate stock to generate separate formulations. The final concentrations of the active compounds were standardized as follows: clove oil (0.5% v/v; emulsified with 0.1% Tween-80), garlic extract (5% v/v), turmeric powder (1% w/v), honey (10% w/v), and vinegar (5% v/v).

Each formulation was vortexed until homogeneous and allowed to rest briefly for air bubble removal. Glass coupons (2–2 cm) were washed, ethanol-cleaned, and dried before coating. A uniform 300  $\mu\text{L}$  layer of each formulation was applied to the surface of the coupons and allowed to dry in an incubator at 40°C (2–4 h). Three independent batches were prepared for each formulation, with at least three coated coupons per batch and corresponding uncoated and alginate-only controls. All coated samples were stored in sterile, labeled Petri dishes at room temperature [18].

**Application of Natural Antimicrobial Coatings on Glass Slides Using One-Side Masking Technique-** To ensure uniform and reproducible coating deposition on glass slides, a one-sided masking technique was employed. Clean microscopy slides (20 x 20 mm) were washed, rinsed with distilled water, disinfected with 70% ethanol, and dried thoroughly. One entire surface of each slide was masked using transparent cello tape to prevent coating deposition on the non-test side. The tape was pressed firmly to eliminate air bubbles and create a complete seal along the edges, leaving the opposite side exposed for coating. Coatings were applied either by dip-coating, immersing the unmasked side in the antimicrobial formulation by spread-coating using 300  $\mu\text{L}$  of formulation distributed evenly across the unmasked surface. After coating, slides were air-dried for 10–15 minutes and further dried in an incubator at 40°C for 30–45 minutes until a stable, non-tacky film formed. The cello tape was then gently removed to reveal a clean, uncoated reverse side. All coated slides were stored individually in sterile, labeled Petri dishes. This standardized masking method ensured precise, uniform, and single-sided coating suitable for accurate antibacterial assessment in subsequent experiments.

**Evaluation of Antimicrobial Activity of Coated Slides Against Individual Pathogens and Mixed Culture-** Assessing the antimicrobial activity of the natural extract-coated slides against *Staphylococcus aureus*, *Serratia marcescens*, *Pseudomonas aeruginosa*, and a mixed bacterial suspension. Each organism was grown overnight and standardized to an  $\text{OD}_{600}$  of 0.1, corresponding to approximately  $10^8$  CFU/mL. A mixed inoculum was prepared by combining equal volumes of each standardized culture. Coated slides, alginate-only slides, and uncoated slides were placed in sterile Petri dishes, and 100  $\mu\text{L}$  of each standardized inoculum was applied to the coated surface and spread evenly. The slides were incubated at 37°C for 1 hour to ensure adequate bacterial contact with the surface. Following exposure, bacterial recovery was performed by

swabbing the entire inoculated area using sterile swabs and transferring the swab into 5 mL of sterile saline. The tubes were vortexed for 10 seconds to release adherent cells, generating recovery suspensions for subsequent OD and plate count analysis. This standardized surface inoculation and recovery process ensured reproducible assessment of the antimicrobial properties of the coated surfaces [19].

**Quantification of Bacterial Survival Using Plate Count Methods-** Quantifying bacterial survival and reduction on coated and uncoated slides using viable plate count techniques. Serial dilutions ( $10^{-1}$  to  $10^{-3}$ ) of each suspension were prepared using sterile saline, and 100  $\mu\text{L}$  from appropriate dilutions was plated onto Nutrient agar. The plates were incubated at 37°C for 24 hours, after which colony counts were taken from plates containing 30–300 colonies. CFU/mL, CFU/cm<sup>2</sup>, log<sub>10</sub> reduction, and percentage reduction were calculated to compare bacterial survival on coated, alginate-only coated surfaces. This plate count approach provided a robust quantitative evaluation of antimicrobial effectiveness, allowing clear differentiation between the performances of various natural extract coatings.

**Comparative Evaluation and Selection of the Most Effective Antimicrobial Coating-** To determine the most effective natural antimicrobial coating, all five alginate-based formulations (clove, garlic, turmeric, honey, and vinegar) were evaluated alongside alginate-only and uncoated control slides under identical experimental conditions. Each slide type was tested against *Staphylococcus aureus*, *Serratia marcescens*, *Pseudomonas aeruginosa*, and a mixed bacterial suspension. Standardized inocula were applied to the coated surfaces, followed by 1-hour contact at 37°C, and bacterial survivors were quantified using viable plate counts to calculate CFU/cm<sup>2</sup>, percent reduction, and log<sub>10</sub> reduction. Results were compared statistically across coatings using ANOVA or non-parametric equivalents, and coatings were ranked based on predefined selection criteria, including  $\geq 90\%$  reduction,  $\geq 1-2$  log<sub>10</sub> reduction, consistent performance across replicates, statistical significance over controls, and broad-spectrum activity against both Gram-positive and Gram-negative organisms. Additional considerations such as durability, cost, and practical applicability in food-contact environments were used to finalize the selection. This comprehensive comparative analysis enabled the identification of the most efficient and industry-relevant natural antimicrobial coating.

## OBSERVATION

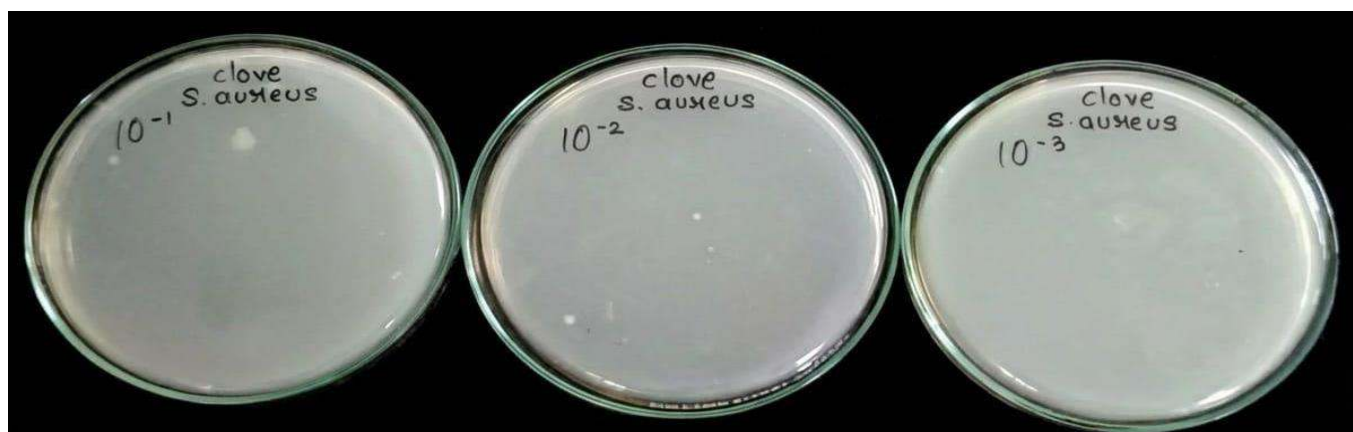


Figure 1A. *Staphylococcus aureus* - Petri plate images showing colony counts of *S. aureus* at serial dilutions ( $10^{-1}$ – $10^{-3}$ ) from clove-alginate film-coated slides



Figure 1B. *Pseudomonas aeruginosa* - Petri plate images of *P. aeruginosa* at serial dilutions ( $10^{-1}$ - $10^{-3}$ ) from clove-alginate film-coated slides, showing THTC growth at lower dilutions



Figure 1C. *Serratia marcescens* - Petri plate images showing *S. marcescens* colonies at serial dilutions ( $10^{-1}$ - $10^{-3}$ ) from clove-alginate film-coated slides



Figure 1D. Mixed colonies - Petri plate images of mixed bacterial colonies at serial dilutions ( $10^{-1}$ - $10^{-3}$ ) from clove-alginate film-coated slides

Table 1. Raw bacterial counts from serial dilutions for slides coated with clove-alginate film

Slide No	Organism	Dilution	(Cfu/0.1 ml)	(Cfu/1 ml)	Mean Cfu/ml
1	<i>S. aureus</i>	$10^{-1}$	15	$150 \times 10^1$	$13.167 \times 10^3$
		$10^{-2}$	8	$80 \times 10^2$	
		$10^{-3}$	3	$30 \times 10^3$	
2	<i>P. aeruginosa</i>	$10^{-1}$	THTC	THTC	$63 \times 10^3$
		$10^{-2}$	63	$630 \times 10^2$	
		$10^{-3}$	NG	NG	
3	<i>S. marcescens</i>	$10^{-1}$	360	$3600 \times 10^1$	$373.34 \times 10^3$
		$10^{-2}$	124	$1240 \times 10^2$	
		$10^{-3}$	96	$960 \times 10^3$	
4	Mixed colony	$10^{-1}$	112	$1120 \times 10^1$	$11.20 \times 10^3$
		$10^{-2}$	NG	NG	
		$10^{-3}$	NG	NG	



Figure 2A. *Staphylococcus aureus* - Petri plate images of *S. aureus* at serial dilutions ( $10^{-1}$ - $10^{-3}$ ) from honey-alginate film-coated slides, showing THTC growth at lower dilutions



Figure 2B. *Pseudomonas aeruginosa* - Petri plate images showing *P. aeruginosa* colony counts at serial dilutions ( $10^{-1}$ - $10^{-3}$ ) from honey-alginate film-coated slides



Figure 2C. *Serratia marcescens* - Petri plate images of *S. marcescens* at serial dilutions ( $10^{-1}$ - $10^{-3}$ ) from honey-alginate film-coated slides, with countable colonies at higher dilution



Figure 2D. Mixed colonies - Petri plate images of mixed bacterial colonies at serial dilutions ( $10^{-1}$ - $10^{-3}$ ) from honey-alginate film-coated slides

Table 2. Raw bacterial counts from serial dilutions for slides coated with Honey-alginate film

Slide No	Organism	Dilution	(Cfu/0.1 ml)	(Cfu/1 ml)	Mean Cfu/ml
1	<i>S. aureus</i>	$10^{-1}$	THTC	THTC	$1400 \times 10^3$
		$10^{-2}$	THTC	THTC	
		$10^{-3}$	140	$1400 \times 10^3$	
2	<i>P. aeruginosa</i>	$10^{-1}$	260	$2600 \times 10^1$	$415.34 \times 10^3$
		$10^{-2}$	180	$1800 \times 10^2$	
		$10^{-3}$	104	$1040 \times 10^3$	
3	<i>S. marcescens</i>	$10^{-1}$	THTC	$THTC \times 10^1$	$860 \times 10^3$
		$10^{-2}$	THTC	$THTC \times 10^2$	
		$10^{-3}$	186	$1860 \times 10^3$	
4	Mixed colony	$10^{-1}$	THTC	THTC	$2120 \times 10^3$
		$10^{-2}$	THTC	THTC	
		$10^{-3}$	212	$2120 \times 10^3$	



Figure 3A. Staphylococcus aureus - Petri plate images of *S. aureus* at serial dilutions ( $10^{-1}$ - $10^{-3}$ ) from garlic-alginate film-coated slides, showing THTC growth at all dilutions



Figure 3B. Pseudomonas aeruginosa - Petri plate images showing *P. aeruginosa* colonies at serial dilutions ( $10^{-1}$ - $10^{-3}$ ) from garlic-alginate film-coated slides



Figure 3C. *Serratia marcescens* - Petri plate images of *S. marcescens* at serial dilutions ( $10^{-1}$ - $10^{-3}$ ) from garlic-alginate film-coated slides, with countable colonies at  $10^{-3}$



Figure 3D. Mixed colonies - Petri plate images of mixed bacterial colonies at serial dilutions ( $10^{-1}$ - $10^{-3}$ ) from garlic-alginate film-coated slides

Table 3. Raw bacterial counts from serial dilutions for slides coated with Garlic-alginate film

Slide No	Organism	Dilution	(Cfu/0.1 ml)	(Cfu/1 ml)	Mean Cfu/ml
1	<i>S. aureus</i>	$10^{-1}$	THTC	THTC	THTC
		$10^{-2}$	THTC	THTC	
		$10^{-3}$	THTC	THTC	
2	<i>P. aeruginosa</i>	$10^{-1}$	THTC	THTC	$820 \times 10^3$
		$10^{-2}$	180	$1800 \times 10^2$	
		$10^{-3}$	128	$1280 \times 10^3$	
3	<i>S. marcescens</i>	$10^{-1}$	THTC	THTC	$1920 \times 10^3$
		$10^{-2}$	THTC	THTC	
		$10^{-3}$	192	$1920 \times 10^3$	
4	Mixed colony	$10^{-1}$	290	$2900 \times 10^1$	$785 \times 10^3$
		$10^{-2}$	246	$2460 \times 10^2$	
		$10^{-3}$	208	$2080 \times 10^3$	



Figure 4A. *Staphylococcus aureus* - Petri plate images of *S. aureus* at serial dilutions ( $10^{-1}$ - $10^{-3}$ ) from turmeric-alginate film-coated slides



Figure 4B. *Pseudomonas aeruginosa* - Petri plate images showing *P. aeruginosa* colonies at serial dilutions ( $10^{-1}$ - $10^{-3}$ ) from turmeric-alginate film-coated slides

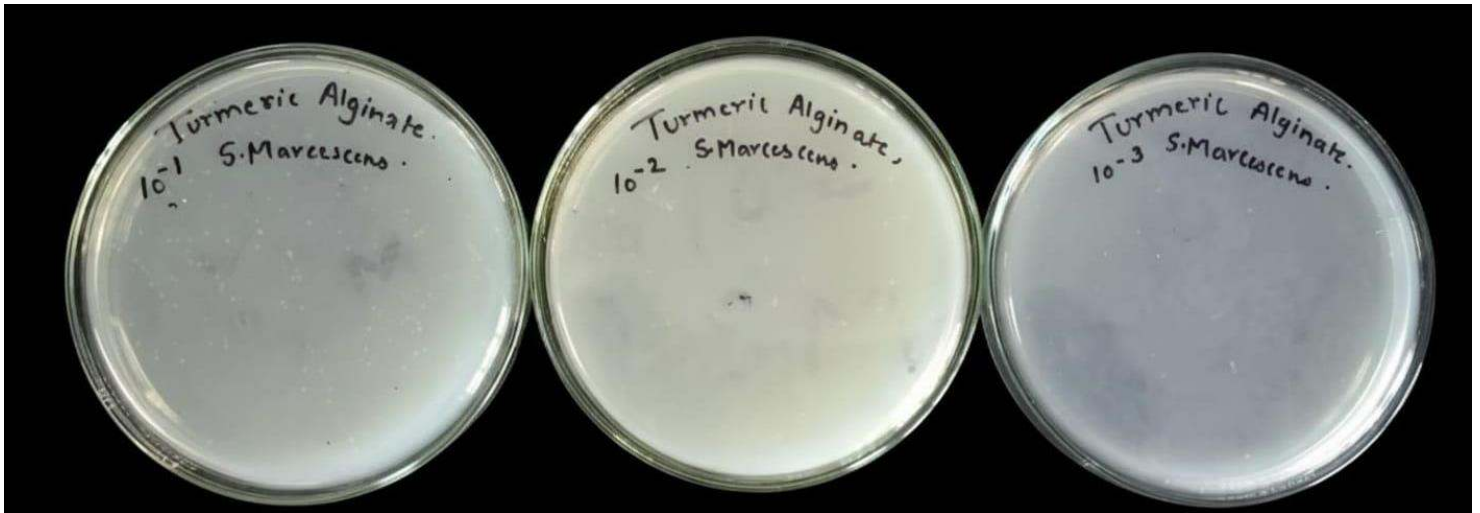


Figure 4C. *Serratia marcescens* - Petri plate images of *S. marcescens* at serial dilutions ( $10^{-1}$ - $10^{-3}$ ) from turmeric-alginate film-coated slides



Figure 4D. Mixed colonies - Petri plate images of mixed bacterial colonies at serial dilutions ( $10^{-1}$ - $10^{-3}$ ) from turmeric-alginate film-coated slides

Table 4. Raw bacterial counts from serial dilutions for slides coated with turmeric-alginate film

Slide No	Organism	Dilution	(Cfu/0.1 ml)	(Cfu/1 ml)	Mean Cfu/ml
1	<i>S. aureus</i>	$10^{-1}$	144	$1440 \times 10^1$	$48.8 \times 10^3$
		$10^{-2}$	72	$720 \times 10^2$	
		$10^{-3}$	6	$60 \times 10^3$	
2	<i>P. aeruginosa</i>	$10^{-1}$	THTC	THTC	$468 \times 10^3$
		$10^{-2}$	186	$1860 \times 10^2$	
		$10^{-3}$	75	$750 \times 10^3$	
3	<i>S. marcescens</i>	$10^{-1}$	176	$1760 \times 10^1$	$115.2 \times 10^3$
		$10^{-2}$	98	$980 \times 10^2$	
		$10^{-3}$	23	$230 \times 10^3$	
4	Mixed colony	$10^{-1}$	THTC	THTC	$690 \times 10^3$
		$10^{-2}$	THTC	THTC	
		$10^{-3}$	69	$690 \times 10^3$	



Figure 5A. *Staphylococcus aureus* - Petri plate images of *S. aureus* at serial dilutions ( $10^{-1}$ - $10^{-3}$ ) from acetic acid-alginate film-coated slides



Figure 5B. *Pseudomonas aeruginosa* - Petri plate images showing *P. aeruginosa* colonies at serial dilutions ( $10^{-1}$ - $10^{-3}$ ) from acetic acid-alginate film-coated slides



Figure 5C. *Serratia marcescens* - Petri plate images of *S. marcescens* at serial dilutions ( $10^{-1}$ - $10^{-3}$ ) from acetic acid-alginate film-coated slides



Figure 5D. Mixed colonies - Petri plate images of mixed bacterial colonies at serial dilutions ( $10^{-1}$ - $10^{-3}$ ) from acetic acid-alginate film-coated slides

Table 5. Raw bacterial counts from serial dilutions for slides coated with Acetic acid –alginate film

Slide No	Organism	Dilution	(Cfu/0.1 ml)	(Cfu/1 ml)	Mean Cfu/ml
1	<i>S. aureus</i>	10 <sup>-1</sup>	THTC	THTC	820 X 10 <sup>3</sup>
		10 <sup>-2</sup>	THTC	THTC	
		10 <sup>-3</sup>	82	820 X 10 <sup>3</sup>	
2	<i>P. aeruginosa</i>	10 <sup>-1</sup>	THTC	THTC	96 X 10 <sup>3</sup>
		10 <sup>-2</sup>	72	720 X 10 <sup>2</sup>	
		10 <sup>-3</sup>	12	120 X 10 <sup>3</sup>	
3	<i>S. marcescens</i>	10 <sup>-1</sup>	THTC	THTC	32 X 10 <sup>3</sup>
		10 <sup>-2</sup>	32	320 X 10 <sup>2</sup>	
		10 <sup>-3</sup>	0	0	
4	Mixed colony	10 <sup>-1</sup>	62	620 X 10 <sup>1</sup>	54.4 X 10 <sup>3</sup>
		10 <sup>-2</sup>	37	370 X 10 <sup>2</sup>	
		10 <sup>-3</sup>	12	120 X 10 <sup>3</sup>	



Figure 6A. *Staphylococcus aureus* - Petri plate images of *S. aureus* at serial dilutions (10<sup>-1</sup>-10<sup>-3</sup>) from alginate film-only coated slides, showing predominantly THTC growth



Figure 6B. *Pseudomonas aeruginosa* - Petri plate images of *P. aeruginosa* at serial dilutions (10<sup>-1</sup>-10<sup>-3</sup>) from alginate film-only coated slides, showing heavy bacterial growth



Figure 6C. *Serratia marcescens* - Petri plate images of *S. marcescens* at serial dilutions (10<sup>-1</sup>-10<sup>-3</sup>) from alginate film-only coated slides, showing THTC growth at all dilutions



Figure 6D. Mixed colonies - Petri plate images of mixed bacterial colonies at serial dilutions ( $10^{-1}$ - $10^{-3}$ ) from alginate film-only coated slides, showing extensive growth

Table 6. Raw bacterial counts from serial dilutions for slides coated with Alginate film Only

Slide No	Organism	Dilution	(Cfu/0.1 ml)	(Cfu/1 ml)	Mean Cfu/ml
1	<i>S. aureus</i>	$10^{-1}$	THTC	THTC	$2600 \times 10^3$
		$10^{-2}$	THTC	THTC	
		$10^{-3}$	260	$2600 \times 10^3$	
2	<i>P. aeruginosa</i>	$10^{-1}$	THTC	THTC	$2680 \times 10^{-3}$
		$10^{-2}$	THTC	THTC	
		$10^{-3}$	268	$2680 \times 10^{-3}$	
3	<i>S. marcescens</i>	$10^{-1}$	THTC	THTC	THTC
		$10^{-2}$	THTC	THTC	
		$10^{-3}$	THTC	THTC	
4	Mixed colony	$10^{-1}$	THTC	THTC	$243.5 \times 10^{-3}$
		$10^{-2}$	67	$670 \times 10^2$	
		$10^{-3}$	42	$420 \times 10^3$	

## CALCULATIONS

### Experimental conditions (same for all samples):

- Recovery volume: 5 mL
- Exposed slide area:  $4 \text{ cm}^2$
- Control: Alginate-only coating

### 1. Alginate-only (Control)

Table 7. Quantitative recovery of viable bacteria from alginate-only coated glass slides expressed as mean CFU/mL, CFU/cm<sup>2</sup>, serving as the control for calculation of log<sub>10</sub> and percentage reduction for all test coatings

Organism	Mean CFU/mL	CFU/cm <sup>2</sup>
<i>S. aureus</i>	$2.6 \times 10^6$	$3.25 \times 10^6$
<i>P. aeruginosa</i>	$2.68 \times 10^6$	$3.35 \times 10^6$
<i>S. marcescens</i>	THTC	THTC
Mixed colony	$2.44 \times 10^5$	$3.04 \times 10^5$

### 2. Clove-alginate coating

Table 8. Antibacterial efficacy of clove-alginate coated glass slides against *S. aureus*, *P. aeruginosa*, *S. marcescens*, and mixed bacterial colonies, expressed as mean CFU/mL, CFU/cm<sup>2</sup>, log<sub>10</sub> reduction, and percentage reduction relative to alginate-only control

Organism	Mean CFU/mL	CFU/cm <sup>2</sup>	Log <sub>10</sub> reduction	% reduction
<i>S. aureus</i>	$1.32 \times 10^4$	$1.65 \times 10^4$	2.29	99.49
<i>P. aeruginosa</i>	$6.30 \times 10^4$	$7.88 \times 10^4$	1.63	97.65
<i>S. marcescens</i>	$3.73 \times 10^5$	$4.67 \times 10^5$	NC	NC
Mixed colony	$1.12 \times 10^4$	$1.40 \times 10^4$	1.34	95.39

### 3. Honey-alginate coating

Table 9. Quantitative evaluation of bacterial survival on honey-alginate coated glass slides showing mean CFU/mL, CFU/cm<sup>2</sup>, log<sub>10</sub> reduction, and percentage reduction in comparison with alginate-only coated control slides

Organism	Mean CFU/mL	CFU/cm <sup>2</sup>	Log <sub>10</sub> reduction	% reduction
<i>S. aureus</i>	$1.40 \times 10^6$	$1.75 \times 10^6$	0.27	46.2
<i>P. aeruginosa</i>	$4.15 \times 10^5$	$5.19 \times 10^5$	0.81	84.5
<i>S. marcescens</i>	$8.60 \times 10^5$	$1.08 \times 10^6$	NC	NC
Mixed colony	$2.12 \times 10^6$	$2.65 \times 10^6$	NR	NR

### 4. Garlic-alginate coating

Table 9. Antibacterial performance of garlic-alginate coated glass slides against different test organisms, expressed as mean CFU/mL, CFU/cm<sup>2</sup>, log<sub>10</sub> reduction, and percentage reduction relative to alginate-only control

Organism	Mean CFU/mL	CFU/cm <sup>2</sup>	Log <sub>10</sub> reduction	% reduction
<i>S. aureus</i>	THTC	THTC	NC	NC
<i>P. aeruginosa</i>	$8.20 \times 10^5$	$1.03 \times 10^6$	0.51	69.2
<i>S. marcescens</i>	$1.92 \times 10^6$	$2.40 \times 10^6$	NC	NC
Mixed colony	$7.85 \times 10^5$	$9.81 \times 10^5$	NR	NR

### 5. Turmeric-alginate coating

Table 10. Quantitative assessment of antibacterial activity of turmeric-alginate coated glass slides against Gram-positive, Gram-negative, and mixed bacterial cultures based on mean CFU/mL, CFU/cm<sup>2</sup>, log<sub>10</sub> reduction, and percentage reduction compared to alginate-only control

Organism	Mean CFU/mL	CFU/cm <sup>2</sup>	Log <sub>10</sub> reduction	% reduction
<i>S. aureus</i>	$4.88 \times 10^4$	$6.10 \times 10^4$	1.73	98.1
<i>P. aeruginosa</i>	$4.68 \times 10^5$	$5.85 \times 10^5$	0.76	82.5
<i>S. marcescens</i>	$1.15 \times 10^5$	$1.44 \times 10^5$	NC	NC
Mixed colony	$6.90 \times 10^5$	$8.63 \times 10^5$	NR	NR

### 6. Acetic acid-alginate coating

Table 11. Comparative antibacterial efficacy of acetic acid-alginate coated glass slides expressed as mean CFU/mL, CFU/cm<sup>2</sup>, log<sub>10</sub> reduction, and percentage reduction relative to alginate-only coated control surfaces

Organism	Mean CFU/mL	CFU/cm <sup>2</sup>	Log <sub>10</sub> reduction	% reduction
<i>S. aureus</i>	$8.20 \times 10^5$	$1.03 \times 10^6$	0.50	68.5
<i>P. aeruginosa</i>	$9.60 \times 10^4$	$1.20 \times 10^5$	1.45	96.4
<i>S. marcescens</i>	$3.20 \times 10^4$	$4.00 \times 10^4$	NC	NC
Mixed colony	$5.44 \times 10^4$	$6.80 \times 10^4$	0.65	77.6

## Quantitative Evaluation of Antimicrobial Performance of Coated Surfaces

### A. *Staphylococcus aureus*

**Table 12.** Organism-wise evaluation of the antibacterial efficacy of natural extract–alginate coatings against *Staphylococcus aureus*, expressed as  $\log_{10}$  reduction, percentage reduction relative to the alginate-only control, statistical significance ( $p < 0.05$ ), and categorized performance level

Coating	$\log_{10}$ Reduction	% Reduction	Statistical significance vs control ( $p < 0.05$ )	Performance level
Alginate-only (control)	0.00	0%	–	No activity
Clove–alginate	2.29	99.5%	Yes	Excellent
Turmeric–alginate	1.73	98.1%	Yes	High
Acetic acid–alginate	0.50	68.5%	Yes	Moderate
Honey–alginate	0.27	46.2%	No	Low

### B. *Pseudomonas aeruginosa*

**Table 13.** Organism-wise evaluation of the antibacterial efficacy of natural extract–alginate coatings against *Pseudomonas aeruginosa*, expressed as  $\log_{10}$  reduction, percentage reduction relative to the alginate-only control, statistical significance ( $p < 0.05$ ), and categorized performance level

Coating	$\log_{10}$ Reduction	% Reduction	Statistical significance vs control ( $p < 0.05$ )	Performance level
Alginate-only (control)	0.00	0%	–	No activity
Clove–alginate	1.63	97.6%	Yes	Excellent
Turmeric–alginate	1.45	96.4%	Yes	High
Acetic acid–alginate	0.76	82.5%	Yes	Moderate
Honey–alginate	0.81	84.5%	No	Moderate

### C. *Serratia marcescens*

**Table 14.** Organism-wise evaluation of the antibacterial efficacy of natural extract–alginate coatings against *Serratia marcescens*, expressed as  $\log_{10}$  reduction, percentage reduction relative to the alginate-only control, statistical significance ( $p < 0.05$ ), and categorized performance level. NC indicates values not calculated due to THTC or non-recoverable counts

Coating	$\log_{10}$ Reduction	% Reduction	Statistical significance vs control ( $p < 0.05$ )	Performance level
Alginate-only (control)	0.00	0%	–	No activity
Clove–alginate	NC	NC	–	Not quantifiable
Turmeric–alginate	NC	NC	–	Not quantifiable
Acetic acid–alginate	NC	NC	–	Not quantifiable
Honey–alginate	NC	NC	–	Not quantifiable

### D. Mixed Bacterial Population

**Table 15.** Organism-wise evaluation of the antibacterial efficacy of natural extract–alginate coatings against mixed bacterial colonies recovered from inoculated surfaces, expressed as  $\log_{10}$  reduction, percentage reduction relative to the alginate-only control, statistical significance ( $p < 0.05$ ), and categorized performance level

Coating	$\log_{10}$ Reduction	% Reduction	Statistical significance vs control ( $p < 0.05$ )	Performance level
Alginate-only (control)	0.00	0%	–	No activity
Clove–alginate	1.34	95.4%	Yes	Excellent
Turmeric–alginate	0.65	77.6%	Yes	Moderate
Acetic acid–alginate	NR	NR	–	Not reliable
Honey–alginate	NR	NR	–	Not reliable

## Comparative performance evaluation and ranking of alginate-based natural antimicrobial coatings

**Table 16.** Integrated performance evaluation and ranking of alginate-based natural antimicrobial coatings based on antibacterial spectrum, statistical significance, and practical applicability

Coating	Broad-spectrum activity	Statistical significance vs control ( $p < 0.05$ )	Practical applicability	Total Score	Rank
Alginate-only (control)	No	–	–	–	–
Clove–alginate	Yes	Yes	High	16	1
Turmeric–alginate	Partial	Yes	Medium	12	2
Acetic acid–alginate	Partial	Yes	Medium	11	3
Honey–alginate	Low	No	Medium	8	4
Garlic–alginate	Low	No	Medium	7	5

Supplementary Data Table for Unified scoring system used for comparative evaluation and ranking of alginate-based natural antimicrobial coatings is given below

### Keynotes

- NC (Not Calculated): Indicates values that could not be determined due to THTC (Too High To Count) colony numbers, absence of countable plates, or non-quantifiable bacterial growth following surface recovery.
- THTC (Too High To Count): Refers to plates exhibiting confluent bacterial growth exceeding the acceptable counting range (30–300 CFU), rendering accurate enumeration unreliable.
- Total Score: Represents the cumulative score assigned to each coating based on predefined performance criteria, including:
  - Antibacterial efficacy measured as  $\log_{10}$  reduction in viable counts.
  - Percentage reduction in bacterial survival relative to the alginate-only control.
  - Broad-spectrum activity, defined as effective inhibition of both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa*) bacteria.
  - Statistical significance of bacterial reduction compared to the alginate-only control ( $p < 0.05$ ).
  - Practical applicability, considering coating stability, ease of preparation, handling feasibility, and suitability for food-contact surface applications.
- Scoring Methodology: Each parameter was scored independently according to the standardized criteria described in Supplementary Table 17, and individual parameter scores were summed to obtain the final total score.

Ranking: Coatings were ranked in descending order of total score, with higher scores indicating superior antimicrobial performance, broader activity spectrum, and greater practical relevance.

**Table 17. Unified scoring system for comparative evaluation of alginate-based antimicrobial coatings, detailing  $\log_{10}$  reduction, percentage reduction, broad-spectrum activity, statistical significance, and practical applicability criteria**

Evaluation parameter	Criterion	Score assigned
Log <sub>10</sub> reduction (per organism)	≥ 2.0	4
	1.0 – 1.99	3
	0.5 – 0.99	2
	0.1 – 0.49	1
	< 0.1, NC, or THTC	0
Percentage reduction	≥ 99%	4
	90 – 98.9%	3
	70 – 89.9%	2
	50 – 69.9%	1
	< 50%	0
Broad-spectrum activity	Effective against both ( <i>S. aureus</i> ) and ( <i>P. aeruginosa</i> )	2
	Effective against only one bacterial group	1
	No consistent activity	0
Statistical significance vs control	Significant (p < 0.05)	2
	Not significant	0
Practical applicability	High (stable coating, feasible for food-contact use)	2
	Medium (moderate stability or handling constraints)	1
	Low (limited usability)	0
Maximum achievable total score	—	16

## RESULTS

The antibacterial performance of alginate-based natural antimicrobial coatings was evaluated against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Serratia marcescens*, and a mixed bacterial population, using alginate-only coated slides as the control. The alginate control showed no measurable antibacterial effect against any tested organism, confirming that observed reductions were attributable to the incorporated natural antimicrobial agents. Among all formulations, clove–alginate coating demonstrated the highest and most consistent antibacterial efficacy. It achieved a 2.29  $\log_{10}$  (99.5%) reduction against *S. aureus* and a 1.63  $\log_{10}$  (97.6%) reduction against *P. aeruginosa*, along with a 1.34  $\log_{10}$  (95.4%) reduction in mixed bacterial populations. These reductions were statistically significant compared to the control (p < 0.05), and clove–alginate was the only coating that exhibited clear broad-spectrum antibacterial activity. Turmeric–alginate coating showed strong activity against *S. aureus* (1.73  $\log_{10}$ ; 98.1%) but moderate efficacy against *P. aeruginosa* (0.76  $\log_{10}$ ; 82.5%). While statistically significant reductions were observed, activity against mixed bacterial populations was inconsistent, resulting in partial broad-spectrum classification. Acetic acid–alginate coating displayed notable antibacterial activity against *P. aeruginosa* (1.45  $\log_{10}$ ; 96.4%) but limited efficacy against *S. aureus* (0.50  $\log_{10}$ ; 68.5%). Moderate reductions were also observed in mixed cultures (0.65  $\log_{10}$ ; 77.6%). This coating achieved statistical significance but exhibited organism-dependent performance. Honey–alginate and garlic–alginate coatings showed comparatively lower and inconsistent antibacterial effects. Neither coating achieved ≥ 1  $\log_{10}$  reduction against both Gram-positive and Gram-negative organisms, and statistical significance over the control was not consistently observed. Garlic–alginate showed THTC values for *S. aureus*, preventing reliable log reduction calculations. Based on predefined scoring criteria integrating  $\log_{10}$  reduction, percentage reduction, broad-spectrum activity, statistical significance, and practical applicability, the coatings were ranked as follows: Clove–alginate > Turmeric–alginate > Acetic acid–alginate > Honey–alginate > Garlic–alginate.

## DISCUSSIONS

The results demonstrate that incorporation of natural antimicrobial agents into a sodium alginate biopolymer matrix significantly enhances surface-level antibacterial activity compared to alginate alone. The superior performance of clove–alginate coating can be attributed to the presence of eugenol, a phenolic compound known to disrupt bacterial cell membranes, denature proteins, and interfere with enzyme systems. The ability of clove–alginate to effectively reduce both Gram-positive and Gram-negative bacteria, as well as mixed microbial populations, highlights its strong potential for real-world food-contact applications. The differential performance observed among coatings underscores the importance of antimicrobial polymer compatibility. Although turmeric possesses well-documented antimicrobial properties, its reduced effectiveness against *P. aeruginosa* may be related to the limited diffusion of curcuminoids through the Gram-negative outer membrane. Similarly, acetic acid–alginate showed enhanced activity against *P. aeruginosa*, likely due to intracellular acidification and metabolic disruption, but exhibited limited activity against *S. aureus*, suggesting that acid-based mechanisms alone may not provide uniform broad-spectrum control when immobilized in a polymer matrix. The relatively low efficacy of honey–alginate and garlic–alginate coatings may be explained by instability or reduced bioavailability of active compounds when incorporated into solid films. For example, allicin from garlic is known to be chemically unstable, and honey's antimicrobial activity relies partly on osmotic effects and hydrogen peroxide generation, which may be diminished in a dried coating format. Importantly, mixed culture experiments revealed that antimicrobial efficacy can be altered by interspecies interactions, emphasizing the necessity of evaluating coatings under conditions that better mimic real-world contamination. The robust performance of clove–alginate under mixed bacterial conditions further supports its suitability for practical application.

## CONCLUSIONS

This study demonstrates that biopolymer-based natural antimicrobial coatings represent a viable and sustainable strategy for enhancing the hygiene of food-contact surfaces. Among the five natural agents evaluated, clove–alginate coating emerged as the most effective, exhibiting statistically significant, broad-spectrum antibacterial activity against Gram-positive, Gram-negative, and mixed bacterial populations. Turmeric–alginate and acetic acid–alginate coatings showed moderate and organism-specific efficacy, while honey–alginate and garlic–alginate coatings were less effective under the tested conditions. Overall, the findings highlight that antimicrobial performance is strongly influenced by the nature of the active compound, its stability, and its interaction with the biopolymer matrix. The results support the potential use of clove–alginate coatings as a safe, eco-friendly, and cost-effective alternative to conventional chemical sanitizers in food processing and handling environments.

## FUTURE PERSPECTIVES

Future studies should focus on optimizing formulation parameters such as antimicrobial concentration, crosslinking density, and coating thickness to maximize antimicrobial durability and controlled release. Long-term performance assessments, including resistance to repeated washing, mechanical abrasion, and extended storage, are essential to evaluate industrial feasibility.

Further investigation into biofilm inhibition, multispecies community dynamics, and coating performance on commonly used food-contact materials such as stainless steel and plastic is recommended. Additionally, regulatory, sensory, and scale-up studies will be critical to facilitate commercial adoption. The integration of natural antimicrobial biopolymer coatings into routine sanitation strategies holds strong potential to reduce chemical sanitizer usage, improve food safety, and support sustainable, consumer-driven hygiene practices in the food industry.

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