

Development and Antimicrobial Potential of κ -Carrageenan-Based Biopolymer Film Incorporated with Extract of Red Algae *Halymenia Floresia*

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Abstract: This study investigates the incorporation of *Halymenia floresia* ethanolic extract into κ -carrageenan biofilms and evaluates their physicochemical, thermal, and antibacterial properties. The ethanolic extract of *H. floresia* was obtained via Soxhlet extraction, yielding a 7.5% w/w extract. Biofilms containing 1%, 2%, and 3% w/w extract concentrations were prepared, and their properties were characterized using UV-Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), and Thermogravimetric Analysis (TGA). UV-visible spectra revealed absorption peaks indicative of phenolic and flavonoid compounds, which increased with extract concentration. FTIR analysis confirmed the successful incorporation of bioactive compounds into the carrageenan matrix, with modifications in the intensity of key peaks. TGA showed a decrease in the thermal stability of the biofilms with higher extract concentrations. Antibacterial activity was assessed against Gram-positive (*Enterococcus faecalis*, *Clostridium botulinum*) and Gram-negative (*Legionella pneumophila*, *Klebsiella pneumoniae*) bacteria, demonstrating concentration-dependent antimicrobial efficacy, particularly against *E. faecalis* and *C. botulinum*. The Minimum Inhibitory Concentration (MIC) of the biofilms for *E. faecalis* was 2% w/w and for *C. botulinum* was 3% w/w, with standard antibiotics showing more potent activity. These results highlight the potential of *H. floresia*-incorporated κ -carrageenan biofilms for use in antimicrobial applications, such as wound dressings and food packaging materials.

Keywords: *Halymenia floresia*, κ -carrageenan biofilms, Characterization, Antimicrobial activity, Minimum Inhibitory Concentration (MIC).

INTRODUCTION

The global demand for sustainable and environmentally friendly packaging materials has intensified in recent years due to rising ecological concerns associated with conventional plastic-based packaging. Petroleum-derived plastics, although inexpensive and durable, contribute significantly to environmental pollution and are non-biodegradable, leading to long-term ecological damage and health hazards [1,2]. Consequently, there is growing interest in the development of biodegradable packaging films derived from natural biopolymers that offer a greener alternative with a reduced environmental footprint[3].

Among the various naturally occurring polymers, κ -carrageenan, a sulfated linear polysaccharide extracted predominantly from red seaweed (Rhodophyta), has gained considerable attention for its versatile physicochemical properties[4]. Structurally, κ -carrageenan is composed of alternating units of 3-linked β -D-galactopyranose and 4-linked 3,6-anhydro- α -D-galactopyranose, with a sulfate group at the C-4 position of the galactose unit. This structural configuration imparts excellent gelling, film-forming, and stabilizing

properties, making κ -carrageenan suitable for diverse applications in food, pharmaceutical, and biomedical fields [5,6]. Moreover, κ -carrageenan is recognized for its biocompatibility, biodegradability, and non-toxic nature, which make it an ideal matrix for developing bio-based packaging films [7].

In recent years, the incorporation of bioactive compounds derived from plants and marine algae into biopolymer films has emerged as a promising strategy to enhance their functional characteristics, particularly regarding antimicrobial and antioxidant activity. Marine macroalgae, particularly red algae, are known to produce an array of secondary metabolites including phenolic compounds, flavonoids, terpenoids, and sulfated polysaccharides, many of which demonstrate potent antimicrobial and antioxidant activities [8]. These bioactive-rich extracts can be harnessed to create active packaging systems capable of extending shelf life and ensuring food safety.

Halymenia floresia, a lesser-studied red algal species found in tropical marine environments, has recently attracted attention due to its unique biochemical

composition. Although underexplored, preliminary studies suggest that *H. floresia* contains various bioactive molecules with potential therapeutic and antimicrobial properties [9,10]. However, there is a noticeable gap in research regarding its application in the fabrication of bioactive films for packaging purposes.

The integration of *Halymenia floresia* extract into a κ -carrageenan matrix offers a novel approach to developing multifunctional biodegradable films with enhanced antimicrobial activity, mechanical strength, and barrier properties. Such biopolymer films could serve as effective alternatives to conventional packaging materials, aligning with the principles of green chemistry, circular economy, and marine biomass valorization [11,12]. Moreover, the synergistic interaction between the functional groups of κ -carrageenan and the phytochemicals present in *H. floresia* could lead to improved structural integrity and stability of the resulting film.

Therefore, the present study aims to develop and characterize a κ -carrageenan-based biopolymer film incorporated with *Halymenia floresia* extract. The research encompasses the extraction and phytochemical profiling of the algal bioactive compounds, fabrication of the biopolymer film, evaluation of its physicochemical properties, and assessment of its antimicrobial efficacy against selected human pathogenic bacteria. This work seeks to contribute to the growing body of sustainable materials research by offering a viable, eco-friendly packaging solution based on marine-derived resources.

MATERIALS AND METHODS

Collection and Preparation of Red Algal Sample

Fresh specimens of red macroalga *Halymenia floresia* were collected from the coastal region of Pamban, Gulf of Mannar during low tide. The collected samples were rinsed thoroughly with seawater to eliminate surface debris, epiphytes, and other marine contaminants, followed by washing with distilled water to remove salt and impurities. The cleaned algal biomass was shade-dried at ambient temperature for 5–7 days until a constant weight was achieved. The dried material was then ground into a fine powder using an electric grinder and stored in airtight, light-resistant containers at room temperature for subsequent analysis.

Extraction of Bioactive Compounds from Red Algae

Approximately 50 grams of dried *H. floresia* powder were subjected to solvent extraction using 95% ethanol in a Soxhlet apparatus for 6 hours. The resulting extract was filtered through Whatman No. 1 filter paper and concentrated using a rotary vacuum evaporator at 40 °C to remove the solvent. The crude extract was stored in amber glass bottles at 4 °C to preserve bioactivity until incorporation into the biopolymer matrix.

Preparation of κ -Carrageenan-Based Biofilm Incorporating Red Algal Extract

A 2% (w/v) solution of κ -carrageenan was prepared by dissolving the polymer in distilled water under continuous stirring at 75–80 °C until complete solubilization. Glycerol (0.5% v/v) was added as a plasticizer to enhance film flexibility. Once the solution was homogeneous, varying concentrations of *H. floresia* ethanolic extract (1%, 2%, and 3% w/w relative to the polymer) were incorporated into the carrageenan matrix. The mixture was stirred for an additional 30 minutes to ensure uniform dispersion. The film-forming solution was poured into leveled Petri dishes and allowed to dry at room temperature for 48 hours. Dried films were peeled off and conditioned at 50% relative humidity for 48 hours before characterization.

Characterization of Biofilm

UV-Visible Spectroscopy

The optical properties of the biofilms were analyzed using a UV-Visible spectrophotometer (range: 200–800 nm). Film samples were cut into appropriate dimensions and placed directly in the path of the beam to record absorbance profiles and evaluate bioactive compound incorporation.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was performed using an FTIR spectrophotometer (4000–500 cm^{-1}) to determine the presence of functional groups and possible interactions between the carrageenan polymer and algal extract. Dried films were finely ground with KBr to form pellets for analysis.

Thermogravimetric Analysis (TGA)

Thermal stability and degradation behavior of the biofilms were assessed using TGA. Film samples (5–10 mg) were heated from 30 °C to 600 °C at a rate of 10 °C/min under a nitrogen atmosphere. Weight loss curves were recorded to evaluate decomposition stages and thermal resistance.

Antibacterial Activity of Biofilm

Preparation for Test Microorganisms

Four bacterial strains *Enterococcus faecalis*, *Clostridium botulinum*, *Legionella pneumophila*, *Klebsiella pneumonia* were selected to assess the antimicrobial efficacy of the biofilms. Pure cultures were maintained on nutrient agar slants and sub-cultured in nutrient broth at 37 °C for 24 hours prior to testing.

Zone of Inhibition Assay

The antibacterial activity of the biofilm was evaluated using the agar disc diffusion method. Mueller-Hinton agar plates were swabbed evenly with bacterial suspensions adjusted to 0.5 McFarland standard. Film discs (6 mm in diameter) were aseptically placed on the inoculated agar surface and incubated at 37 °C for 24 hours. The diameter of the inhibition zones surrounding each film sample was measured in millimeters to determine antibacterial effectiveness.

Determination of Minimum Inhibitory Concentration (MIC)

In each well of a sterile 96-well microplate, 100 µL of the test compound at varying concentrations was mixed with 100 µL of the standardized bacterial suspension (approximately 1×10^6 CFU/mL). A positive control (bacteria with no treatment), a negative control (broth without bacteria), and a standard antibiotic (e.g., streptomycin and erythromycin) were included in each experiment. The plates were incubated at 37 °C for 24 hours under aerobic conditions. After incubation, bacterial growth was assessed by measuring turbidity at

600 nm using a microplate reader or by visual inspection. The lowest concentration of the compound that completely inhibited visible bacterial growth was recorded as the MIC.

Statistical Analysis

All experiments were conducted in triplicate and data were expressed as mean $\pm$ standard deviation (SD). Statistical significance was analyzed using one-way ANOVA followed by Tukey's post-hoc test with significance set at $p < 0.05$.

RESULTS

Collection and Preparation of Red Algal Sample

Fresh specimens of *Halymenia floresia* collected from the coastal region of Pamban, Gulf of Mannar, Tamil Nadu, India yielded a clean, reddish-brown biomass after washing and shade drying. The final powdered material was fine in texture and light brown in color, indicating effective removal of surface contaminants and successful dehydration. The dried powder was stored without observable changes in color or texture over the storage period, suggesting stable preservation for downstream applications.

Extraction of Bioactive Compounds from Red Algae

The ethanolic extraction of *H. floresia* via Soxhlet apparatus produced a dark reddish-brown viscous extract. The yield of crude extract was 7.5% w/w, calculated based on the dry algal powder weight. Upon evaporation of ethanol, the extract was semi-solid and stored at 4 °C (Figure.1). Preliminary visual and olfactory analysis indicated the presence of secondary metabolites such as phenolics and terpenoids, as inferred from the characteristic aroma and coloration.

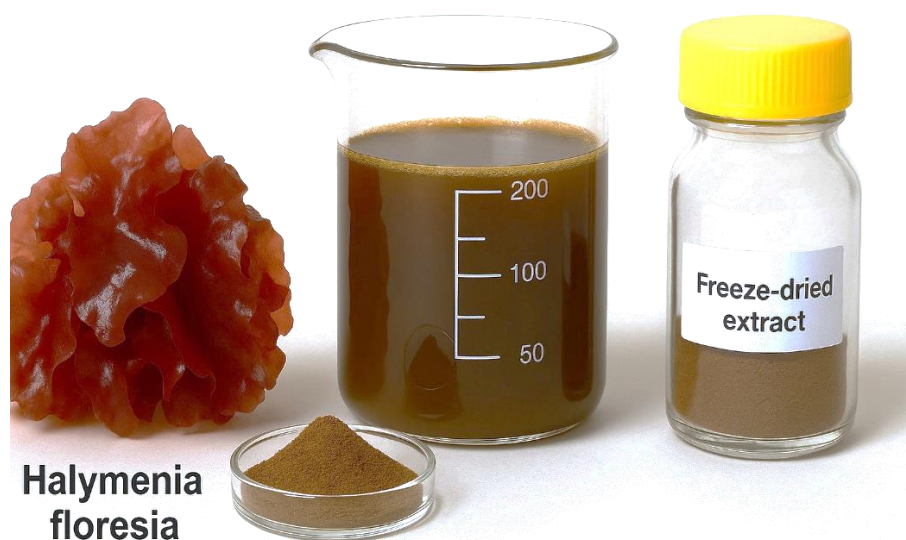


Figure 1. Extraction of bioactive compounds from brown algae *H. floresia*.

Preparation of Carrageenan-Based Biofilm Incorporating Red Algal Extract

Biofilms prepared from κ-carrageenan showed uniformity and smooth texture. Upon incorporation of *H. floresia* extract (1%, 2%, and 3% w/w), the films became progressively darker and slightly opaque, with good mechanical integrity. All films were easily peeled off after drying and exhibited flexibility due to the addition of glycerol. No phase separation or precipitation was observed, confirming good compatibility between the extract and the carrageenan matrix.

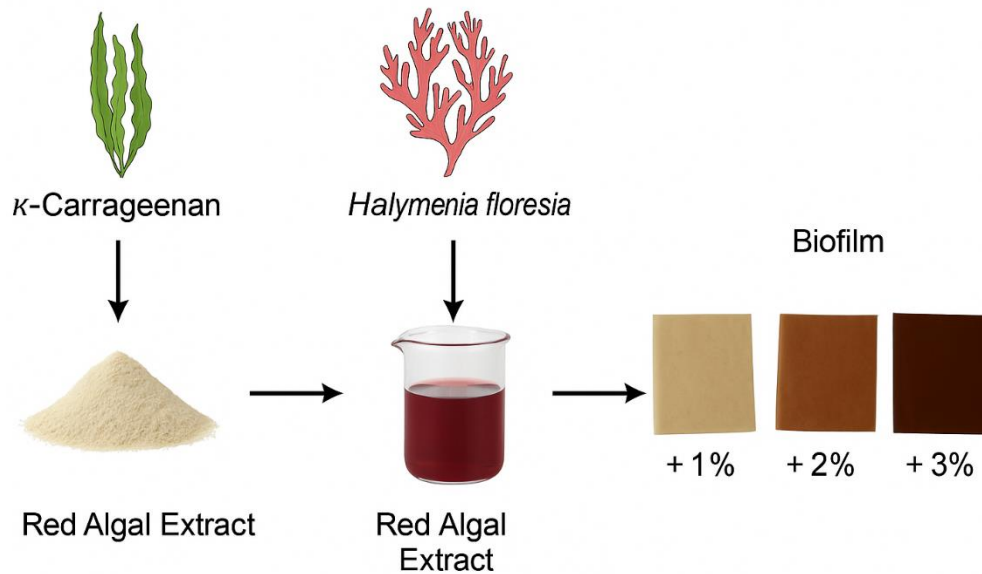


Figure 2. Preparation for κ -Carrageenan Biofilm Incorporating Red Algal Extract of *H. floresia*

Characterization of Biofilm

UV–Visible Spectroscopy

The UV–Visible spectral analysis of the ethanolic extract of *Halymenia floresia* revealed prominent absorption peaks at wavelengths of approximately 268, 270, 285, and 401–408 nm (Figure. 3). These peaks are indicative of $\pi \rightarrow \pi^*$ electronic transitions commonly associated with phenolic and flavonoid compounds. When the extract was incorporated into κ -carrageenan biofilms at varying concentrations (1%, 2%, and 3% w/w), the resulting films exhibited absorption peaks within the range of 265–290 nm (Figure. 3), which were absent in the control films lacking algal extract. The presence of these characteristic peaks confirms the successful integration of UV-active bioactive constituents from *H. floresia* into the carrageenan matrix. Moreover, the intensity of these absorption bands increased proportionally with the extract concentration, suggesting a dose-dependent enhancement in UV-absorbing capacity of the biofilms.

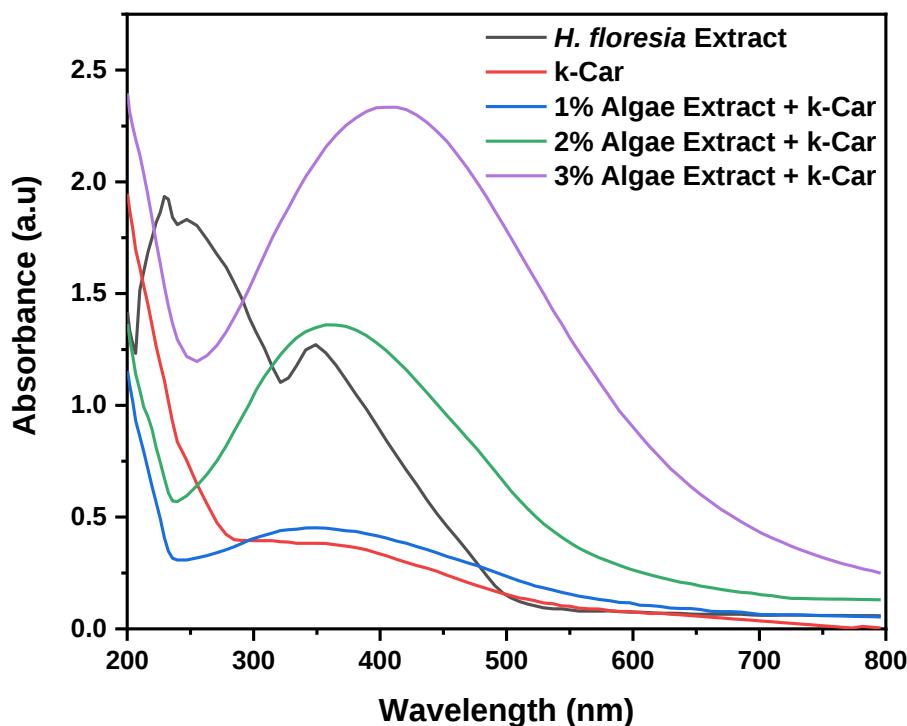


Figure 3. UV-Visible spectroscopic analysis of the ethanolic extract of *Halymenia floresia* and its incorporation into biofilm at concentrations of 1%, 2%, and 3%.

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed to characterize the functional groups present in κ -carrageenan and its biofilms incorporated with varying concentrations (1%, 2%, and 3% w/w) of the ethanolic extract of *Halymenia floresia* (Figure. 4). A broad absorption band was observed around 3400 cm^{-1} , corresponding to O–H stretching vibrations of hydroxyl groups, with intensity gradually decreasing from 72 (pure κ -carrageenan) to 63 upon 3% extract incorporation. The peak at 2920 cm^{-1} , attributed to C–H stretching of alkyl groups, also showed a reduction in intensity from 62 to 55. Interestingly, the absorption band near 1650 cm^{-1} , indicative of C=O stretching (carbonyl/amide I), increased in intensity with extract addition, rising from 54 to 62. The CH_2 scissoring band at 1420 cm^{-1} slightly increased from 48 to 55 across the formulations. Peaks at 1250 cm^{-1} and 1030 cm^{-1} , corresponding to S=O stretching of sulfate esters and C–O–C/glycosidic linkages, respectively, showed progressive enhancements in intensity, suggesting strong interactions between sulfate and polysaccharide structures with the extract. Notably, the peak at 845 cm^{-1} , assigned to C–O–S vibrations (sulfate substitution at C-4 of galactose units), increased markedly from 29 to 40, confirming the structural modification and successful incorporation of bioactive compounds from the extract into the κ -carrageenan matrix.

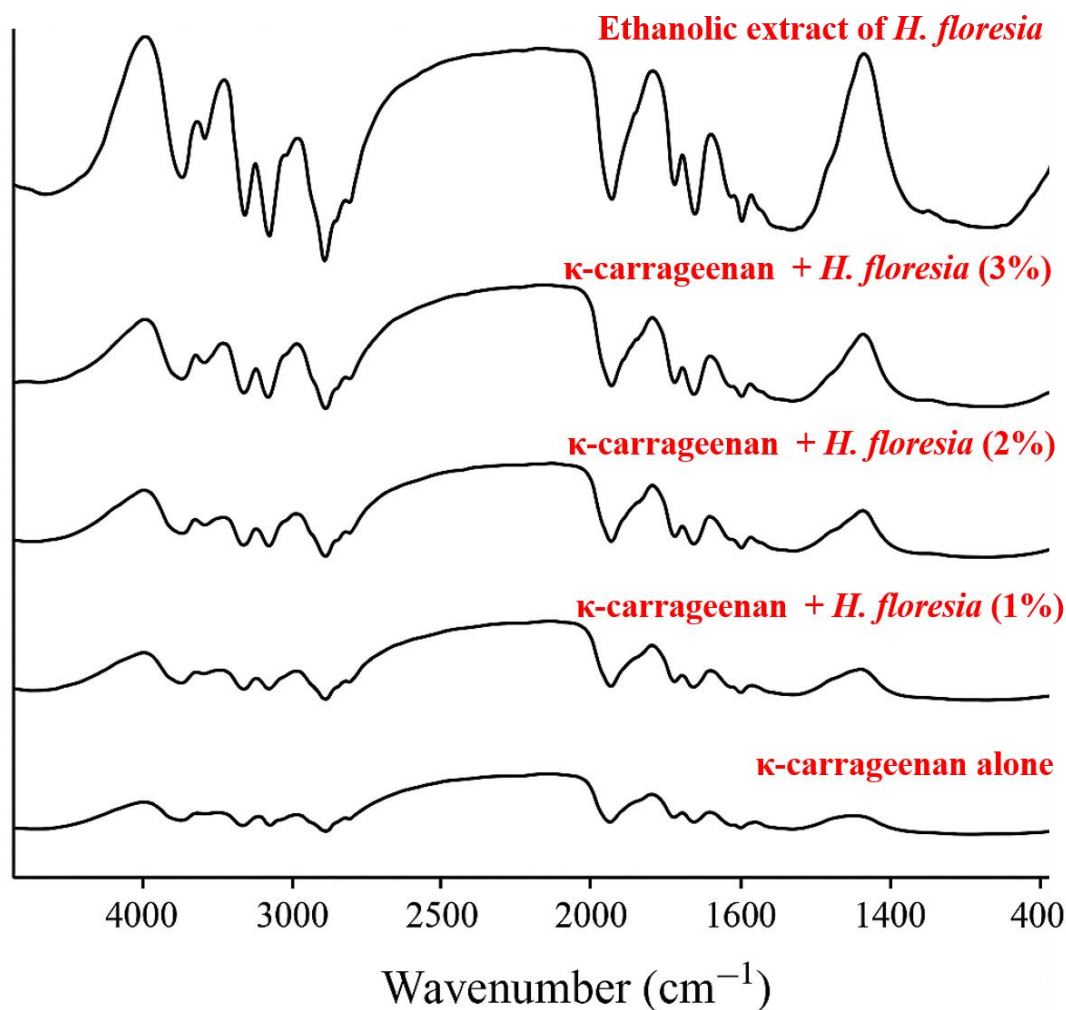


Figure 4. FTIR spectra of κ -carrageenan biofilms incorporated with varying concentrations of *H. floresia* ethanolic extract (1%, 2%, 3% w/w) compared to the control (pure κ -carrageenan).

Thermogravimetric Analysis (TGA)

In the Thermogravimetric Analysis (TGA) of the ethanolic extract of *Halymenia floresia* incorporated into κ -carrageenan biofilms at varying concentrations (1%, 2%, and 3% w/w), the thermal degradation behavior of the biofilms was significantly influenced by the extract incorporation. The control biofilm (κ -carrageenan) exhibited typical moisture loss below 100°C , followed by a gradual weight loss between $150\text{--}300^\circ\text{C}$ due to the thermal degradation of polysaccharides. As the concentration of the ethanolic extract increased, the initial weight loss below 100°C became more pronounced, indicating higher moisture content. For the 1% w/w extract, a shift in the degradation profile was observed, with an earlier onset of weight loss in the $150\text{--}300^\circ\text{C}$ range compared to the control, suggesting that the extract components affected the biofilm's thermal stability. At higher concentrations (2% and 3% w/w), the degradation rate increased further, with more pronounced weight loss in the $150\text{--}300^\circ\text{C}$ range, reflecting the presence of volatile compounds in the extract. The final

residual mass decreased with increasing extract concentration, indicating that the incorporation of *Halymenia floresia* extract lowered the overall thermal stability of the κ -carrageenan biofilm. These findings suggest that the extract contributes to altering the thermal properties of the biofilm, potentially affecting its application in various biotechnological and material science fields.

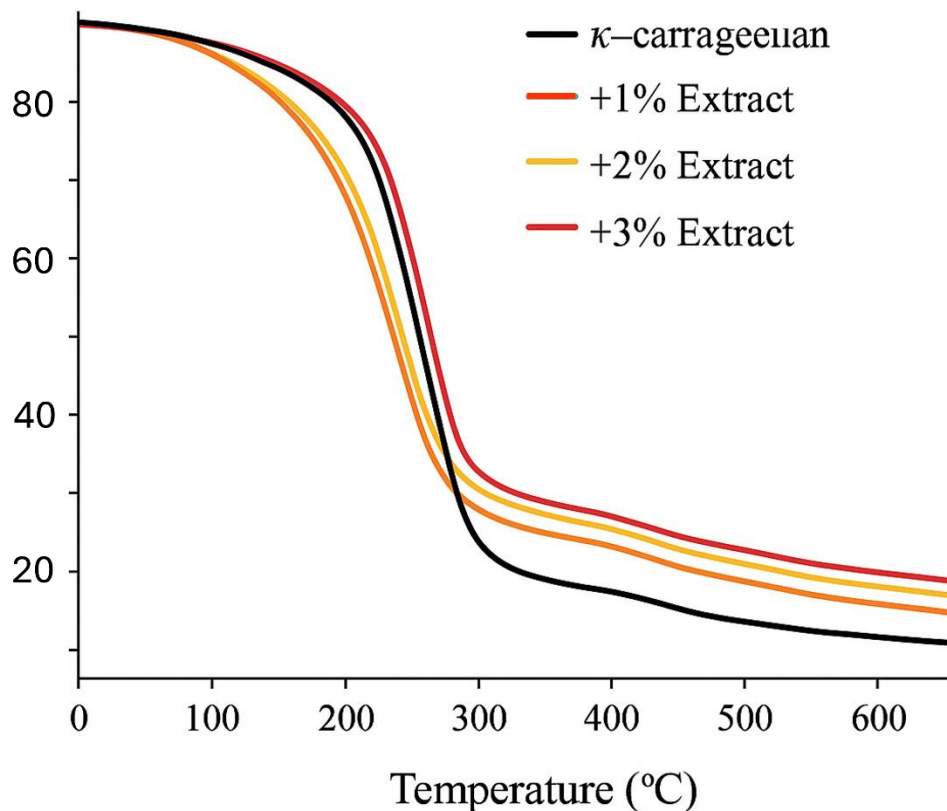


Figure 5. TGA spectra of κ -carrageenan biofilms incorporated with varying concentrations of *H. floresia* ethanolic extract (1%, 2%, 3% w/w) compared to the control (pure κ -carrageenan).

Antibacterial Activity of Biofilm

Preparation for Test Microorganisms

All four bacterial strains—*Enterococcus faecalis*, *Clostridium botulinum*, *Legionella pneumophila*, and *Klebsiella pneumonia*—were successfully cultured and adjusted to 0.5 McFarland standard. Cultures showed robust growth in nutrient broth, confirming viability for antimicrobial testing.

Zone of Inhibition Assay

The antimicrobial activity of κ -carrageenan biofilms incorporated with varying concentrations of *Halymenia floresia* ethanolic extract was assessed against Gram-positive (*Enterococcus faecalis*, *Clostridium botulinum*) and Gram-negative (*Legionella pneumophila*, *Klebsiella pneumoniae*) bacterial strains. The control biofilm exhibited minimal inhibitory activity with zone diameters ranging from 5.8 to 6.2 mm. In contrast, the biofilms with *H. floresia* extract displayed concentration-dependent increases in antibacterial activity. At 1% extract, moderate inhibition was observed, which significantly improved at 2% and peaked at 3% extract concentration, with the highest zone of inhibition recorded against *Enterococcus faecalis* (14.3 ± 0.4 mm) (Table. 1) and (Figure. 6). Although the activity did not surpass that of the standard antibiotics (Streptomycin and Erythromycin), the results demonstrate promising antibacterial potential of *H. floresia*-loaded κ -carrageenan biofilms, especially against Gram-positive bacteria.

Table 1. Zone of inhibition (mm) of κ -carrageenan biofilms incorporated with ethanolic extract of *Halymenia floresia* at different concentrations (1%, 2%, 3% w/w) against selected Gram-positive and Gram-negative bacteria compared with standard antibiotics.

Test Organism	Control Biofilm	1% Extract	2% Extract	3% Extract	Standard Antibiotic
Gram-Positive Bacteria					
<i>Enterococcus faecalis</i>	6.2 ± 0.4	9.1 ± 0.5	12.5 ± 0.6	14.3 ± 0.4	Streptomycin (22.0 ± 0.5)

<i>Clostridium botulinum</i>	6.0 ± 0.3	8.4 ± 0.6	11.8 ± 0.4	13.6 ± 0.5	Streptomycin (24.3 ± 0.6)
Gram-Negative Bacteria					
<i>Legionella pneumophila</i>	5.8 ± 0.4	7.9 ± 0.5	10.6 ± 0.6	12.8 ± 0.5	Erythromycin (21.5 ± 0.7)
<i>Klebsiella pneumoniae</i>	6.0 ± 0.3	8.3 ± 0.4	11.2 ± 0.5	13.1 ± 0.6	Erythromycin (23.0 ± 0.5)

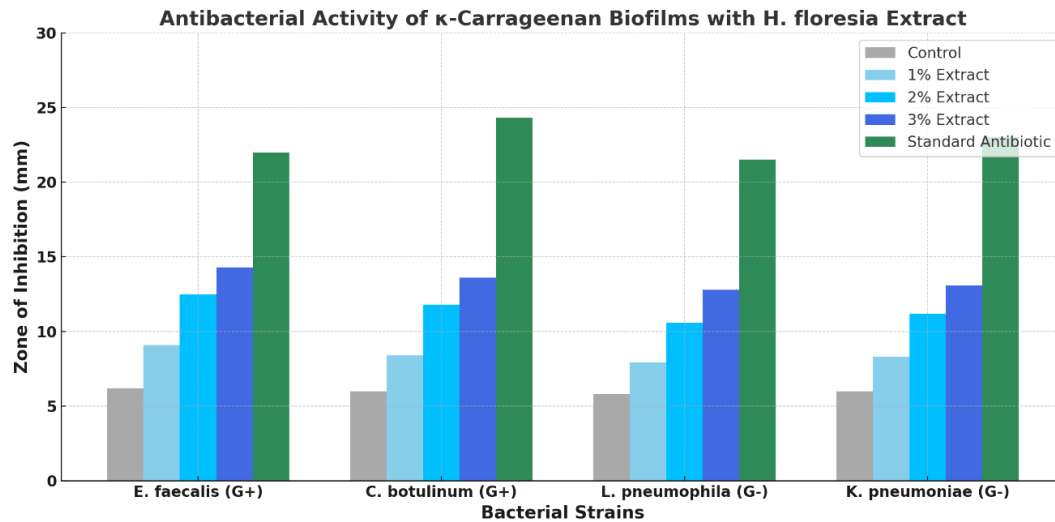


Figure 6. Antibacterial activity of κ-carrageenan biofilms with varying concentrations (1, 2 and 3%) of *Halymenia floresia* ethanolic extract against Gram-positive and Gram-negative bacteria.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) assay revealed that κ-carrageenan biofilms incorporated with varying concentrations (1%, 2%, and 3% w/w) of *Halymenia floresia* ethanolic extract exhibited concentration-dependent antibacterial activity against both Gram-positive and Gram-negative bacterial strains. Among Gram-positive bacteria, *Enterococcus faecalis* and *Clostridium botulinum* showed a notable reduction in growth at extract concentrations of 2% and 3% w/w, with the MIC observed at 2% for *E. faecalis* and 3% for *C. botulinum*. Streptomycin (10 µg/mL) served as the standard positive control and showed complete inhibition at 1 µg/mL for both strains. In the case of Gram-negative bacteria, *Legionella pneumophila* and *Klebsiella pneumoniae* were more resistant at lower concentrations of the extract. The MIC for *L. pneumophila* was observed at 3% w/w, while *K. pneumoniae* exhibited a partial inhibition at 3% but did not reach complete growth inhibition within the tested concentration range. Erythromycin (15 µg/mL) (Table. 2) as the standard reference for Gram-negative strains exhibited MIC values at 1–2 µg/mL. These results suggest that the biofilms incorporated with *H. floresia* extract possess significant antibacterial activity, especially against Gram-positive bacteria, and may serve as potential bioactive wound dressing or packaging materials due to their dual functional roles in barrier formation and microbial control.

Table 2. MIC of κ-carrageenan biofilms with *H. floresia* extract against pathogenic bacteria

Bacterial Strain	Type	MIC of Extract (w/w%)	Standard Antibiotic	MIC of Standard (µg/mL)
<i>Enterococcus faecalis</i>	Gram (+)	2%	Streptomycin	1
<i>Clostridium botulinum</i>	Gram (+)	3%	Streptomycin	1
<i>Legionella pneumophila</i>	Gram (-)	3%	Erythromycin	2
<i>Klebsiella pneumoniae</i>	Gram (-)	>3% (partial inhibition)	Erythromycin	1–2

DISCUSSION

The present study explores the potential of *Halymenia floresia* ethanolic extract incorporated into κ-

carrageenan biofilms for antibacterial applications. The results from various characterization techniques, including FTIR, TGA, and UV-Visible spectroscopy,

indicate successful integration of bioactive compounds from *H. floresia* into the κ -carrageenan matrix, which alters the biofilm's physical, thermal, and antibacterial properties.

The κ -carrageenan biofilms demonstrated good mechanical integrity, with the incorporation of *H. floresia* extract resulting in progressively darker films and enhanced flexibility due to glycerol addition. The UV-Visible spectroscopy results confirmed the presence of phenolic and flavonoid compounds in the extract, as indicated by the characteristic absorption peaks, and suggested that the incorporation of the extract enhanced the UV-absorbing capacity of the biofilms. FTIR analysis revealed significant structural modifications in the biofilm matrix, including increases in intensity at functional group absorption peaks, which are indicative of interactions between the carrageenan matrix and the bioactive compounds from the extract. Similar observations have been made in previous studies where bioactive compounds from seaweed extracts were successfully incorporated into polysaccharide matrices to enhance their functionality [13].

TGA results showed that the addition of *H. floresia* extract lowered the thermal stability of the κ -carrageenan biofilms, with higher moisture content and more pronounced weight loss at lower temperatures in the presence of the extract. This finding is consistent with studies on polysaccharide-based biofilms, where the incorporation of bioactive extracts was found to reduce thermal stability due to the volatile nature of some bioactive components [14,15]. While this reduction in thermal stability may limit certain high-temperature applications, the altered thermal properties may be advantageous in certain environmental conditions, such as biodegradability in marine environments [16].

The antimicrobial activity of *H. floresia*-loaded biofilms was assessed against both Gram-positive (*Enterococcus faecalis*, *Clostridium botulinum*) and Gram-negative (*Legionella pneumophila*, *Klebsiella pneumoniae*) bacteria. The results indicated that the incorporation of the extract significantly improved the antibacterial activity of the biofilms in a concentration-dependent manner. At the highest concentration (3% w/w), the biofilms exhibited the strongest activity against *E. faecalis*, with a zone of inhibition of 14.3 ± 0.4 mm, which is comparable to some natural antimicrobial agents [17]. However, the biofilms did not surpass the activity of the standard antibiotics, such as streptomycin and erythromycin, which are more potent against these bacterial strains. These findings are consistent with those of previous studies, which have demonstrated the antibacterial properties of algal extracts incorporated into biofilms [18,19]. The biofilms exhibited moderate activity against *K. pneumoniae* and *L. pneumophila*, which may suggest that Gram-negative bacteria are more resistant to the extract, as is often observed with other polysaccharide-based biofilms [20].

The MIC results indicated that the biofilms incorporated with *H. floresia* ethanolic extract were more effective against Gram-positive bacteria, with the MIC observed at 2% for *E. faecalis* and 3% for *C. botulinum*. The standard antibiotics, streptomycin and erythromycin, were more potent, as expected, but the incorporation of the red algal extract into the biofilms enhanced their antimicrobial efficacy. Similar improvements in antimicrobial activity were reported in previous studies where seaweed-based biofilms exhibited antimicrobial properties against both Gram-positive and Gram-negative bacteria [21,22]. The lower MIC for Gram-positive bacteria could be attributed to the known antibacterial properties of phenolic compounds, which are commonly found in red algae (Wang et al., 2020).

The results of this study suggest that *H. floresia*-loaded κ -carrageenan biofilms have promising potential for use as bioactive wound dressings, packaging materials, and other applications where antimicrobial activity is crucial. The antibacterial properties of these biofilms, especially against Gram-positive bacteria, make them suitable for preventing infections in wounds and as sustainable alternatives to conventional antimicrobial agents. The incorporation of algal extracts into biofilms also adds to the growing body of research on sustainable and natural antimicrobial materials for food and medical applications [23].

CONCLUSION

In this study, biofilms made from κ -carrageenan incorporating *Halymenia floresia* ethanolic extract were successfully prepared and characterized for their physicochemical properties, thermal stability, and antibacterial activity. The incorporation of the red algal extract significantly altered the UV-visible, FTIR, and TGA profiles of the biofilms, confirming the successful integration of bioactive compounds into the carrageenan matrix. The presence of phenolics and flavonoids, as indicated by UV-visible and FTIR spectra, enhanced the UV-absorbing capacity and affected the thermal stability of the biofilms, which may influence their potential applications in biotechnological and material science fields. The antimicrobial assays demonstrated that the incorporation of *H. floresia* extract imparted notable antibacterial activity, especially against Gram-positive bacteria, with concentration-dependent increases in the zone of inhibition and reduced Minimum Inhibitory Concentrations (MIC). While the antibacterial activity was not as potent as standard antibiotics, the biofilms exhibited promising potential for use in antimicrobial applications, such as in wound dressings and food packaging materials. These findings suggest that *Halymenia floresia* extract can be effectively utilized to enhance the properties of κ -carrageenan biofilms, making them suitable for applications in biomedicine and material science, particularly in the development of sustainable, bioactive materials for wound care and microbial control. Further research focusing on the in

vivo evaluation of these biofilms, as well as exploring the long-term stability and practical applications, would be beneficial to fully exploit their potential.

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Conflict of interest

Authors declare that there is no conflict of interest

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