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Dual-action antimicrobial and antioxidant kojic acid-grafted kefiran biopolymer via enhanced iron chelation

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ABSTRACT

This study presents a cost-effective and straightforward synthesis for a functionalized polysaccharide, kefiran, by covalently grafting it with kojic acid to obtain KK. The new KK material demonstrated a significant morphological change, forming more compact aggregates than unmodified kefiran, as observed through scanning electron microscopy (SEM) and Dynamic Light Scattering (DLS). Thermogravimetric analysis (TGA) proved that the KK derivative has a greater ability to chelate iron, an essential element for bacterial growth. The most significant findings were the material's enhanced biological properties. While unmodified kefiran had moderate antimicrobial activity against some Gram-positive bacteria, the functionalized KK material showed a dramatic improvement, especially against Gram-negative pathogens like *E. coli* and *P. aeruginosa*. This enhanced activity is attributed to a cooperative effect between the two components: kefiran's disruption of cell membranes and kojic acid's action in chelating iron. Furthermore, the KK derivative displayed a notable increase in its antioxidant activity and high cellular compatibility following 24 h of exposure. These results highlight a promising strategy for developing new, sustainable biopolymer-based materials with dual antimicrobial and antioxidant properties, suitable for a wide range of applications from food packaging to advanced biomedical devices.

1. Introduction

Growing environmental concerns have sparked a rising interest in biopolymers, such as polysaccharides, for innovative applications in the pharmaceutical and packaging industries [1–3]. These materials, derived from renewable sources, offer intrinsic properties like biodegradability, biocompatibility, and non-toxicity, making them highly suitable for numerous applications and helping to reduce the accumulation of non-degradable substances [4,5].

The stability of products, whether pharmaceuticals or food, is a primary concern. Factors like oxidation, moisture, and microbial contamination can compromise the therapeutic activity of active ingredients, shorten food shelf life, and degrade nutritional and organoleptic properties [6,7]. To combat these issues, there's been a surge in the development of innovative materials that not only protect the product but also control its release or impart additional functionalities [8–11].

In this context, polymeric films, thin continuous layers of material, are particularly relevant. They can be used as protective coatings for oral solid forms, as matrices for modified-release active ingredients, or as components of active and biodegradable dressings [12]. In the broader packaging sector, these coatings act as a barrier to extend food shelf life by protecting products from external agents [13].

Among the various polymers, polysaccharides stand out for their unique properties, such as biocompatibility and biodegradability. In particular, materials like alginic acid (from marine algae) [14,15], chitosan (from crustacean exoskeletons) [16], and kefiran (a polysaccharide derived from milk fermentation) [17] are highly promising for developing functionalized films.

Kefiran has emerged as an extremely versatile biopolymer with a wide range of applications [18–20], not just in the pharmaceutical industry but also in the food and cosmetic sectors. Its unique properties, such as biocompatibility, biodegradability, and its inherent antimicrobial, antioxidant, and even antitumor functions, make it an ideal

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candidate to replace synthetic and less sustainable materials [21,22].

In the food industry, kefiran is highly valued for its ability to improve product stability and quality. It's used as a thickening, stabilizing, and emulsifying agent, and as a fat substitute to create low-calorie products. Its film-forming ability makes it an excellent choice for active food packaging, where it acts as a protective barrier while simultaneously extending shelf life due to its antimicrobial properties [23,24].

In this context, natural antimicrobials such as bacteriocins have been extensively investigated as natural weapons to improve food safety and preservation, offering a complementary strategy to polysaccharide-based coating [25]. Extending beyond food applications, in the biomedical and pharmaceutical fields, kefiran is being explored for its therapeutic potential. It's been studied for its ability to promote wound healing, protect epithelial cells, and act as a potential agent in reducing hypertension and cholesterol [26,27]. Its biocompatible nature makes it a promising material for developing drug delivery systems, where it can serve as a matrix for the controlled release of active ingredients, or for creating hydrogels and scaffolds for tissue engineering [28,29]. Its resistance to enzymatic degradation in the gastrointestinal tract also allows it to function as a prebiotic, promoting the growth of beneficial gut bacteria [21]. The ability of these materials to combine protective functions with other specific activities is key to creating next-generation products. To develop new coatings with these properties, it's crucial to establish easy, fast, and low-cost synthesis methods for their preparation and functionalization, ensuring their large-scale use is economically viable.

A highly effective strategy to enhance the biological profile of these biopolymers is the incorporation of iron-chelating moieties. Iron is a vital micronutrient for nearly all bacterial pathogens [30], playing a critical role in DNA synthesis, respiration, and biofilm formation; thus, its sequestration represents a potent non-traditional antimicrobial mechanism [31]. Previous research has successfully explored this approach by functionalizing polysaccharides with various chelating agents. For instance, grafting catechol groups or EDTA-like molecules onto chitosan and alginate backbones has been shown to significantly inhibit bacterial growth by depriving pathogens of essential metal ions [32,33]. Furthermore, more advanced systems have been developed using phytic acid on nanocellulose to create anti-biofouling surfaces [34], or starch-based hydrogels functionalized with dithiocarbamates for environmental and protective applications [35]. Additionally, materials incorporating hydroxypyridinone units or specialized siderophore mimics, such as deferoxamine-grafted matrices, have demonstrated superior efficacy in disrupting the metabolic pathways of Gram-negative bacteria [36–38]. These strategies highlight the potential of using metal-complexing agents to engineer dual-action systems that combine the structural integrity of the polymer with nutrient deprivation.

This work aims to combine the properties of kefiran with those of kojic acid, both from natural sources. Kojic acid possesses significant chelating properties [39–41], and we leverage its capability to chelate iron, an element essential for bacterial growth. The functionalization of the polysaccharide with kojic acid has, therefore, significantly improved the antimicrobial and antioxidant properties of kefiran, opening new avenues for the creation of innovative and sustainable protective materials.

2. Experimental

2.1. General information

All the required chemicals were purchased from Merck and VWR. Precoated aluminum sheets (silica gel 60 F254, Merck) were used for thin-layer chromatography (TLC) and visualized under UV light. ^{1}H NMR spectra were recorded at 300 K on Varian UNITY Inova using D_2O as the solvent at 500 MHz for ^{1}H NMR. Chemical shift (δ) values were given in ppm.

2.2. Chemistry

2.2.1. Extraction of kefiran

The polysaccharide matrix kefiran was extracted from dehydrated kefir grains purchased from Natural Probio. The extraction procedure was slightly modified from that reported in the literature [42,43]. In brief, 5 g samples of kefir grains were mechanically broken up using a mortar and left in 50 mL of boiling water for 2 h. After centrifugation at 1500 rpm for 15 min, the supernatant was precipitated by adding two volumes of cold ethanol relative to the water and left at −20 °C overnight. The precipitate was dried in an oven at 65 °C for 12 h, yielding 200 mg of white solid.

2.2.2. Synthesis of KK

KK has been synthesized by the nucleophilic substitution reaction of kefiran on the chloride of the chloro kojic acid in the presence of Et_3N [15]. In particular, chloro kojic acid (252 mg, 1.57 mmol), kefiran (42 mg), and Et_3N (328 μL, 2.35 mmol) were solubilized in dry DMF (4 mL) in a round-bottom flask. The reaction mixture was heated to 40 °C and maintained at this temperature under stirring overnight. After cooling to room temperature, the solvent was removed by rotary evaporation, and the precipitate was washed with acetone (2 mL × 3). Finally, the product was dialyzed (1000 Da) for 12 h and dried under vacuum to obtain 44 mg of pure product.

2.3. Infrared spectroscopy

Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (FTIR-ATR) analyses were conducted using an FTIR Agilent Cary 630 equipped with an ATR sampling module. Thin films of the samples were applied to the ATR crystal and pressed gently. The results were derived from 512 scans acquired in the 4000–500 cm^{-1} range with a resolution of 2 cm^{-1} at room temperature.

2.4. Thermogravimetric analysis

Thermal gravimetric analysis (TGA) was used to study the thermal behavior under 1 atm of prepurified nitrogen, with a heating rate of 10 °C/min, in the temperature range of 50–900 °C.

2.5. Scanning electron microscopy (SEM)

The synthesized material's morphology was analyzed by SEM using a Scanning electron microscopies were performed by using a Focused ion beam/scanning electron microscopy (SEM/FIB) Versa™ 3D LoVac DualBeam™ in secondary electron mode using a 5 keV electron beam. Before analysis, all samples were sputtered with 5 nm of Au to ensure proper conductivity during measurements.

2.6. Evaluation of mean particle size and polydispersity index

To evaluate the mean particle size (Z-ave) and polydispersity index (PdI) of kefiran and KK, they were solubilized/suspended in water (1 mg/mL) and analyzed using Photon Correlation Spectroscopy (PCS) with a Zetasizer Nano S90 instrument (Malvern Instruments, Malvern, UK). The instrument was set with a detection angle of 90 °C and a 4 mW He-Ne laser operating at 633 nm, at a temperature of 25 °C. Three sets of measurements were used in the sample analysis, and the mean size ± standard deviation (SD) was reported as the result.

2.7. Antibacterial activity

The antibacterial activity of the tested substances was evaluated by determining the Minimum Inhibitory Concentration (MIC) using the broth microdilution method. The bacterial strains used in this study included *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC

25213, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, and *Enterococcus faecalis* ATCC 29212. All strains were grown overnight on Muller-Hinton (MH) agar at 37 °C under aerobic conditions. A 0.5 McFarland standard suspension was prepared from overnight cultures and diluted to a final concentration of approximately 1.0×10^6 CFU/mL. Each tested compound was dissolved in sterile water and further diluted in ISO-Sensitest broth to prepare the stock solution. Serial two-fold dilutions were then performed to obtain final concentrations ranging from 8000 μg/mL to 250 μg/mL. An aliquot of the bacterial suspension was added to each well, resulting in a final bacterial concentration of approximately 1.0×10^5 CFU/mL. Positive control wells contained only the bacterial suspension, while negative control wells contained only sterile ISO-Sensitest broth. Microplates were incubated at 37 °C for 24 h. MIC values were determined as the lowest concentration at which no visible bacterial growth was observed.

2.8. Cell cultures and cell viability assay

Hep-2 CCL-23 ™ cells were obtained from the American Type Culture Collection (Manassas, VA, USA). Cell line was maintained in EMEM (Eagle's Minimum Essential Medium) ATCC 30–2003 ™ containing 2 mM L-glutamine, supplemented with 10 % fetal bovine serum (FBS, Gibco, Cat. No. 10082147), 100 U/mL penicillin, and 100 U/mL streptomycin (Gibco, Cat. No. 15070063) at a humidified 37 °C incubator providing 5 % CO_2. To evaluate the cytotoxic effects, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was assessed as previously reported [15]. Briefly, cells were seeded in 96-well plates at 1×10^3 cells/well. After 24 h of treatment with 1 mg/mL of KK, 100 μL of MTT solution (0.5 mg/mL) was added to each well. After 3 h of incubation, formazan crystals were dissolved in 150 μL of DMSO. OD was measured at a wavelength of 570 nm with the use of a spectrophotometer for microplates (BioTek Microplate Reader - Synergy HTX). Three tests were performed and the results were expressed as mean ± SD.

2.9. DPPH free radicals scavenging activity

Antioxidant activity of the extracted kefiran samples was assessed by DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radicals scavenging activity [44]. Solutions of kefiran and KK samples were prepared in deionized water at a concentration of 0.1 mg/mL. Subsequently, 3 mL of the samples were mixed with 1 mL DPPH methanolic solution (0.1 mM). The samples were shaken and kept in a dark room at ambient temperature (25 °C) for 30 min. Then, the absorbance intensity of the samples was read at 517 nm using a UV–visible spectrophotometer (Jasco V-730 spectrophotometer). The percentage of DPPH free radicals scavenging activity was obtained by the following Eq. 1:

$$\textbf{\textit{DPPH scavenging activity}}\ (\%) = \left(\frac{\boldsymbol{A_{DPPH}} - \boldsymbol{A_S}}{\boldsymbol{A_{DPPH}}}\right) \times \mathbf{100} \qquad (1)$$

where A_{DPPH} shows the absorbance of the DPPH solution without sample, and A_S is the absorbance of the DPPH solution with kefiran or KK.

3. Results and discussion

3.1. Synthesis and characterization

The material synthesis strategy of KK (Fig. 1) was designed based on our previous work in which the hydroxyl groups of alginic acid were derivatized in the same way with chloro kojic acid [15]. Through ^{1}H NMR analysis, it was possible to verify the covalent modification by chloro kojic acid on the polysaccharide chain of kefiran. In fact, Fig. S1 and Fig. S2 show the ^{1}H NMR spectra of the extracted kefiran and KK, respectively, from which we can see, in addition to the typical signals of the polysaccharide skeleton [45], the signals relating to the protons of kojic acid at 6.55 and 8.22 ppm [15].

Fig. 2 shows the FTIR spectra of the extracted kefiran (black line) and the new KK material (red line). The spectrum of the unmodified kefiran confirms the presence of the typical polysaccharide structure, in fact we note: the broad peak between 3000 and 3500 cm^{-1}, attributed to the OH stretching of the polysaccharide structure, while the broad area between 800 and 1200 cm^{-1}, considered the characteristic area of carbohydrates, is attributed to carbohydrate rings and side groups, including C-O-C, C-OH, and C-H groups [46]. In addition to these, the KK spectrum (Fig. 2, red line) shows signals from the stretching of the carbonyl group C═O at 1647 cm^{-1}, relating to the presence of kojic acid, and also at 1203 cm^{-1}, the new signal relating to the stretching of the new vinyl ether group, which highlights the success of covalent functionalization [15].

Functionalization with kojic acid led to a significant morphological change in the material. As shown in Fig. 3, the SEM images reveal a clear

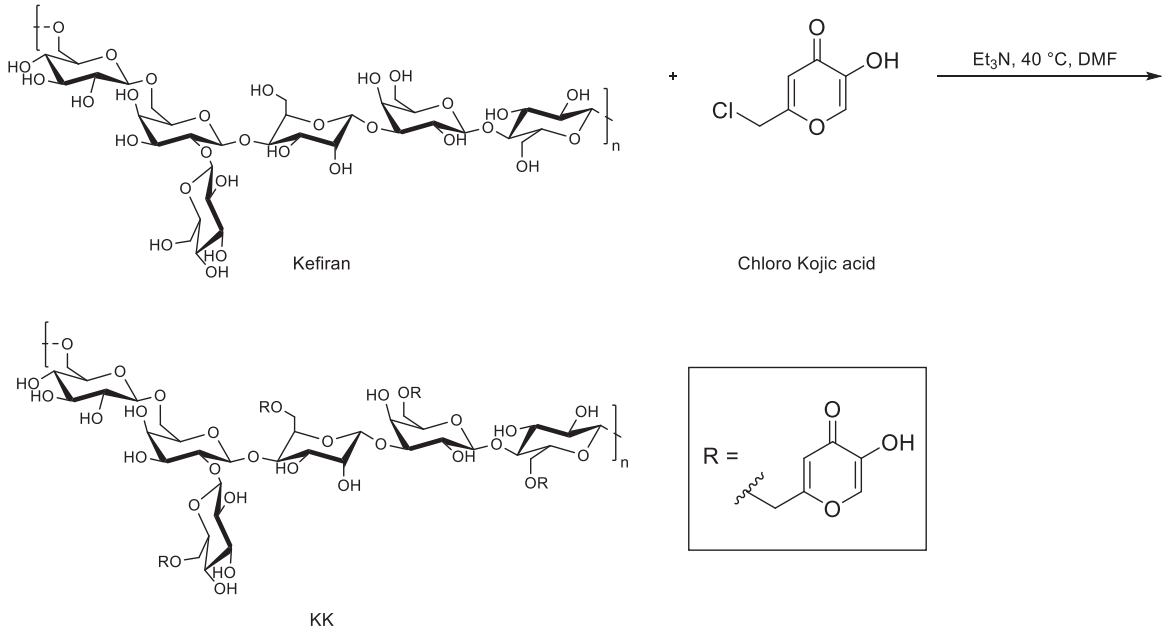

Fig. 1. Reaction scheme for synthesizing KK material.

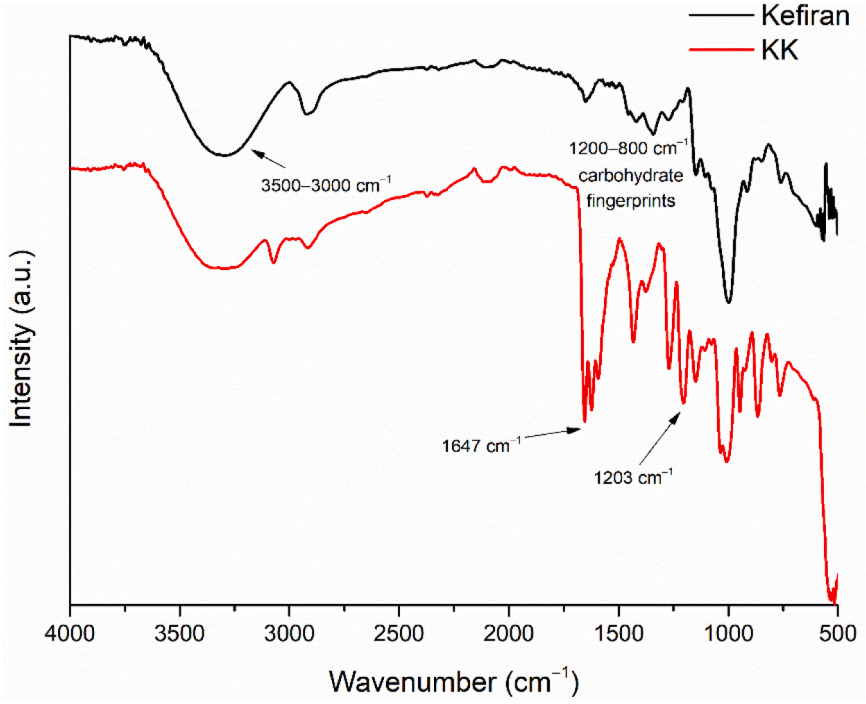

Fig. 2. Stacked IR spectra of kefiran (black line) and KK (red line).

transition: while the unmodified kefiran (Fig. 3a) exhibits a characteristic porous and interconnected network, the KK derivative (Fig. 3b) presents a smoother and more compact surface finish. This loss of porosity and the transition toward a more uniform surface is a direct consequence of the covalent grafting of kojic acid confirmed by NMR and IR. The introduction of the kojic acid moieties likely alters the interchain hydrogen bonding of the polysaccharide, forcing a tighter macromolecular arrangement [47,48]. This suggests that the functionalization does not merely occur on the surface but deeply influences the structural organization and the self-assembly properties of the kefiran chains.

This aspect is also evident from the analyses of the average size (Z-ave) and polydispersity index (PdI) reported in Table 1. Kefiran shows aggregates in solution with a Z-ave of 564.7 nm, which increase following covalent modification with kojic acid. In fact, KK forms much larger aggregates in solution with a Z-ave of 1460 nm.

The thermal properties of kefiran and KK were evaluated by thermogravimetric analysis (TGA) (Fig. 4). Kefiran shows a residue at 900 °C of 3.5 % (Fig. 4 black line), while KK shows 5.8 % (Fig. 4 red line). The same experiment was performed after immersing the two samples in a 10 % wt $FeCl_3$ solution, which was stirred for 30 min and then centrifuged and rinsed with deionized water. After air drying for 24 h, TGA analyses were performed. From the curves shown in Fig. 4, we can see that kefiran-Fe has a residue at 900 °C of 16.2 % (Fig. 4 blue line), while KK-Fe has a residue of 20.1 %, which highlights KK's greater ability to chelate iron due to the presence of kojic acid.

To obtain a direct measurement of the amount of Fe^{3+} complexed by KK, UV-Vis spectroscopy for the analysis of iron in drinking water was adapted to our case (https://documents.thermofisher.com/TFS-Assets/CAD/Application-Notes/uv–vis–spectroscopy-drinking-water-quality-an1217-en.pdf). The calibration curve and corresponding UV spectra are shown in Figures S3 and S4. To quantify the capacity of Fe^{3+} complexed by KK, 1 mL of KK 1 mg/L was added to 1 mL of a 2 mg/L Fe^{3+} solution and stirred for one hour. Afterwards, the solution was centrifuged, and the supernatant was treated to determine the amount of Fe^{3+} remaining uncomplexed (see Fig. S6). From the UV spectrum obtained, we can assume that the amount of Fe^{3+} remaining is 0.6 mg/L and therefore KK was able to chelate the remaining 1.4 mg, or about 70 %.

3.2. Antibacterial and antioxidant activity

The antimicrobial activity of kefiran, both alone and in combination with kojic acid, was evaluated against five clinically relevant bacterial strains (Table 2). When administered alone, kefiran exhibited moderate activity against *Staphylococcus aureus* (MIC = 250 μg/mL) and weak activity against *Enterococcus faecalis* (MIC = 500 μg/mL), while no inhibitory effect was observed against *Escherichia coli*, *Klebsiella pneumoniae*, or *Pseudomonas aeruginosa* at the tested concentrations (MIC >8000 μg/mL). Remarkably, the co-administration of kefiran with kojic acid resulted in a significant improvement in antimicrobial efficacy across all tested strains. In particular, the combination exhibited activity against *E. coli* (MIC = 250 μg/mL) and *K. pneumoniae* and *P. aeruginosa* (MIC = 1000 μg/mL), which were previously unresponsive to kefiran alone. Moreover, the MIC value against *E. faecalis* dropped from 500 μg/mL to 62.5 μg/mL, indicating a nearly eightfold increase in potency. These findings suggest a highly effective dual-action interaction between kefiran and kojic acid. While kefiran may exert its activity through interference with membrane integrity or nutrient uptake, kojic acid is known for its iron-chelating properties, which can disrupt bacterial metabolism by depriving pathogens of essential micronutrients. The observed enhancement may therefore result from the complementary mechanisms of action of the two compounds, one affecting membrane structure and the other targeting intracellular metabolic pathways. Overall, the data support the hypothesis that combining polysaccharide-based antimicrobials with iron-chelating agents may offer a promising strategy for overcoming the limited spectrum of activity of natural polymers such as kefiran, particularly against Gram-negative pathogens.

From a functional standpoint, the MIC values obtained for the kefiran–kojic acid combination reflect growth inhibition arising from the convergence of multiple bacterial stress responses. In line with previous reports on bioactive polysaccharides, growth suppression measured as MIC may result from envelope-associated perturbations,

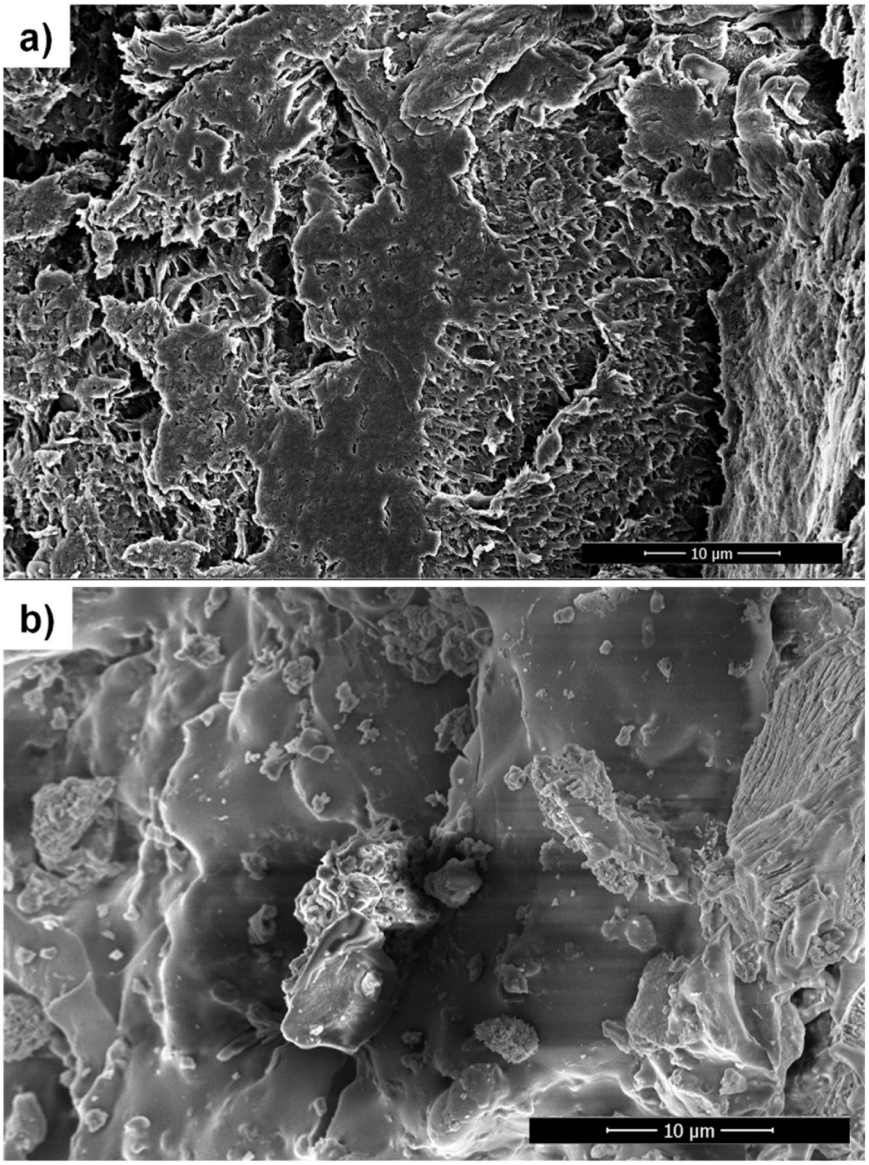

Fig. 3. SEM images of kefiran (a) and KK (b).

Table 1
Mean size (Z-ave) and polydispersity index (PdI) of kefiran and KK.

Sample	Z-ave (nm) ±SD	PDI ±SD
Kefiran	564.7 ± 22.89	0.823 ± 0.03
KK	1460 ± 108.1	0.839 ± 0.23

including altered membrane permeability, interference with nutrient uptake, and impairment of biofilm-related processes, all of which ultimately translate into reduced bacterial proliferation [49].

Concurrently, the incorporation of kojic acid introduces an iron-chelating functionality that is known to enhance bacterial growth limitation. Iron restriction represents a well-established antimicrobial vulnerability, as iron is essential for respiration, DNA synthesis, and the activity of numerous metabolic enzymes. Limiting iron availability has been shown to suppress bacterial growth and virulence, thereby reinforcing the reduction in MIC values observed when iron-chelating agents are combined with polymeric scaffolds [50].

The superior biological performance of the KK material is highlighted in Table 3, where its antimicrobial activity is compared with

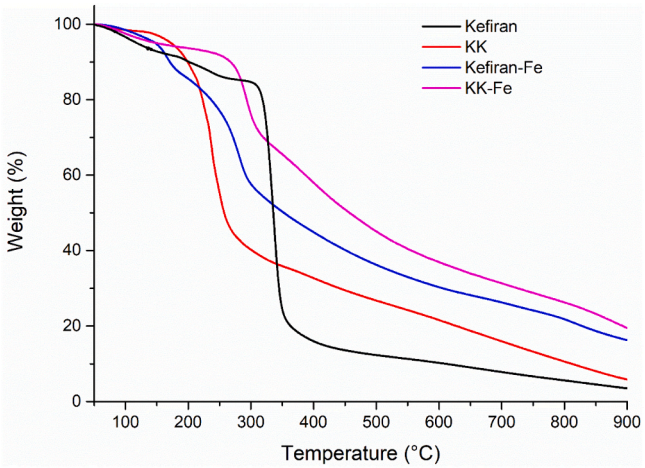

Fig. 4. Thermograms of Kefiran (black line), KK (red line), Kefiran in the presence of Fe (Kefiran-Fe blue line), and KK in the presence of Fe (KK-Fe purple line).

Table 2
MIC values of the compounds (Kefiran and KK) expressed as μg/mL.

Bacterial Strain	Kefiran	KK
E. coli	> 8000	250
S. aureus	250	250
K. pneumoniae	> 8000	1000
P. aeruginosa	> 8000	1000
E. faecalis	500	62.5

Table 3
Comparison of the antibacterial activity of bio-and chelating-based materials against different bacterial strains.

	MIC (mg/mL)				
Gram-positive and Gram-negative bacteria	Pectin-alginate [51]	Piperazine derivatives of chitosan [52]	AK [15]	HNTs–kojic acid [39]	KK (This work)
S. aureus	—	0.008–8.1	1.5	3.0	0.25
E. faecalis	—	0.5–8.1	1.5	3.0	0.06
P. aeruginosa	—	> 8.1	6.0	—	1.00
E. coli	0.25	4.0–8.1	1.5	3.0	0.25
K. pneumoniae	—	—	6.0	3.0	1.00

other functionalized biopolymers and chelating systems reported in the literature. Notably, KK exhibits significantly lower MIC values across a broad spectrum of pathogens compared to its predecessor AK (Alginic acid-Kojic acid) and HNTs-kojic acid (halloysite-based kojic acid) composites. While other derivatives like piperazine-chitosan show competitive MICs for Gram-positive bacteria, KK offers a more balanced and potent activity against challenging Gram-negative pathogens, confirming its potential as a high-performance, sustainable antimicrobial agent for biomedical and packaging applications.

The antioxidant activity of kefiran and the KK derivative was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) antiradical activity test [44]. Solutions of 0.1 mg/mL of kefiran and KK were prepared, which showed scavenger activity of 94.6 % and 98.2 %, respectively (see Fig. S4). This data demonstrated that the presence of kojic acid not only improves the antibacterial properties of the material but also increases its antioxidant activity.

3.3. Cell biocompatibility assay

The potential biocompatibility of the KK material was evaluated on human epithelial Hep-2 cells using the MTT assay. Following 24 h of exposure to KK at a concentration of 1 mg/mL, no evident morphological alterations were observed under optical microscopy when compared with untreated control cells (data not shown).

Quantitative analysis of cell viability, measured as mitochondrial metabolic activity, revealed that treatment with KK did not induce a significant reduction in absorbance at 570 nm relative to control cells. Cell viability values in KK-treated sample were comparable to untreated controls, indicating the absence of detectable cytotoxic effects under the experimental conditions tested, as shown in Fig. 5.

Results were reported as mean ± standard deviation from three independent experiments. Overall, these findings demonstrate that KK exhibits high cellular compatibility following 24 h of exposure and does not impair the metabolic activity of Hep-2 cells *in vitro*.

Collectively, the absence of cytotoxic effects supports the biocompatibility of KK and suggests its suitability for further development in applications requiring contact with human cells, including biomedical and functional material-based systems.

4. Conclusions

In this work, we successfully synthesized a novel functionalized

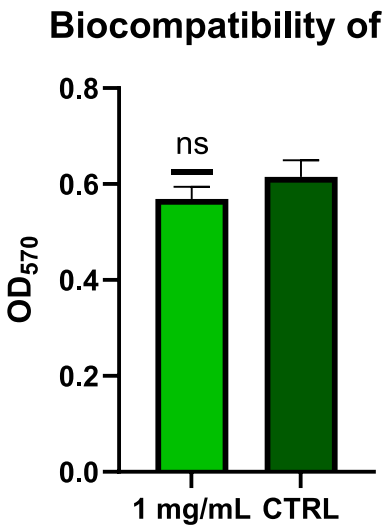

Fig. 5. Cell viability was evaluated by MTT assay after 24 h of exposure to KK at 1 mg/mL and compared with untreated control cells (CTRL). Data are expressed as mean ± SD of three independent experiments. Statistical analysis was performed using Student's *t*-test; no statistically significant differences were observed between treated and control groups (p-value = 0.6021; ns, not significant).

biopolymer, Kefiran-Kojic acid (KK), using a simple and efficient method. The successful covalent grafting of kojic acid onto the kefiran polysaccharide was confirmed through FTIR analysis. It resulted in significant changes in the material morphology and aggregation behavior, as shown by SEM and DLS. The enhanced chelating ability of KK for iron was demonstrated by TGA, which showed a residue increase from 16.2 % in unmodified kefiran to 20.1 % in the KK derivative after exposure to an iron solution.

Most importantly, the functionalization led to a remarkable improvement in the material's biological activity. The functional coupling of kefiran with kojic acid significantly broadened the antimicrobial spectrum, particularly against multidrug-resistant Gram-negative bacteria, which are often challenging to treat. For example, the combination exhibited activity against *E. coli* with a MIC of 250 μg/mL, and a nearly eightfold increase in potency against *E. faecalis*, with the MIC dropping from 500 μg/mL to 62.5 μg/mL. This is attributed to the dual mechanism of action, where the polysaccharide affects bacterial membranes while the chelating agent deprives pathogens of essential micronutrients. Additionally, the functionalized material showed a substantial increase in its antioxidant activity, with a scavenger activity of 98.2 % compared to kefiran's 94.6 % and high cellular compatibility following 24 h of exposure.

These findings provide a strong foundation for the development of highly effective and sustainable biopolymer-based materials. The synthesis strategy presented here is straightforward and adaptable, paving the way for the creation of a new generation of eco-friendly functional materials with applications in active food packaging, biomedical devices, and other fields where enhanced antimicrobial and antioxidant properties are critical. Future work will focus on scaling up the synthesis and testing the material's performance in real-world applications.

CRediT authorship contribution statement

Vincenzo Patamia: Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Formal analysis, Conceptualization. **Antonio Rescifina:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Giuseppe Floresta:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Giulia Sambataro:** Methodology,

Investigation. **Erika Saccullo:** Visualization, Validation, Methodology, Investigation. **Salvatore Furnari:** Methodology, Investigation. **Virginia Fuochi:** Methodology, Investigation. **Furneri Pio:** Writing – review & editing, Resources. **Elena Bruno:** Validation, Resources, Methodology, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mtcomm.2026.114892.

Data availability

Data will be made available on request.

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