Fostering K-carrageenan Hydrogels with the Power of Natural Crosslinkers: A Comparison between Tender Coconut Water and Potassium Chloride for Antibacterial Therapy

Atharva Markale [1, #], Tarun Mateti [2, 3, #], Likhith K. [4, #], S. Supriya Bhatt [5], Rajesh K. M. [6], Vishwanath Managuli [7], Manasa Nune [5], Ritu Raval [6], Pradeep Kumar [8, *], Goutam Thakur [4, *]

[1] *Department of Electrical and Electronics Engineering, Manipal Institute of Technology, Manipal Academy of Higher Education, Manipal–576104, Udupi, Karnataka, India*

[2] *Centre for BioSystems Science and Engineering, Indian Institute of Science, C. V. Raman Road–560012, Bangalore, Karnataka, India*

[3] *Materials Research Centre, Indian Institute of Science, C. V. Raman Road–560012, Bangalore, Karnataka, India*

[4] *Department of Biomedical Engineering, Manipal Institute of Technology, Manipal Academy of Higher Education, Manipal–576104, Udupi, Karnataka, India*

[5] *Manipal Institute of Regenerative Medicine, Manipal Academy of Higher Education, Yelahanka–560065, Bangalore, Karnataka, India*

[6] *Department of Biotechnology, Manipal Institute of Technology, Manipal Academy of Higher Education, Manipal–576104, Udupi, Karnataka, India*

[7] *Department of Mechanical and Industrial Engineering, Manipal Institute of Technology, Manipal Academy of Higher Education, Manipal–576104, Udupi, Karnataka, India*

[8] *Department of Pharmacy and Pharmacology, School of Therapeutic Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg–2193, South Africa*

[#] **Authors contributed equally**; [*] **Correspondences**: Pradeep.Kumar@wits.ac.za, goutam.thakur@manipal.edu; Phone: +91 820-292-4214

Abstract

Hydrogels have emerged as a promising solution to combat infections in various biomedical applications by acting as antibacterial drug carriers, but they lack mechanical strength and must be crosslinked before use. Herein, we investigated whether tender coconut water can be used as a natural alternative to KCl to crosslink κ-carrageenan hydrogels for antibacterial therapy. K-carrageenan hydrogels crosslinked with tender coconut water, KCl, and their combination were fabricated, and their morphology, chemical bonding, compressive strength, water uptake

capacity, degradation resistance, and cytotoxicity were assessed. The results showed that crosslinking with tender coconut water and KCl increased the compressive strength of κ-carrageenan hydrogels by 450%, rendered an excellent water retention capacity, and degraded by just about 5% after 20 days! The scanning electron micrographs show that crosslinking with tender coconut water and KCl compacted the hydrogel morphology with narrow cracks for efficient diffusion, and such were biocompatible when tested against 3T3 cells. The infrared analysis confirmed that diclofenac sodium remained inert when introduced into the hydrogel matrices. Also, the *in-vitro* release behavior and antibacterial activity of the hydrogels were assessed by loading them with diclofenac sodium nanoemulsified to increase hydrophilicity, in which the release of the hydrogels crosslinked with tender coconut water and KCl was steady and sustained. Such hydrogels also showed a unique antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, with the latter much more prominent than the former. These results confirm that crosslinking with tender coconut water and KCl is a superior alternative to KCl for κ-carrageenan hydrogels.

Keywords: natural crosslinkers, hydrogels, diclofenac sodium, antibacterial activity, sustained drug delivery, biocompatible materials

1. Introduction

Hydrogels are polymer networks that can possess high mechanical strength through crosslinking. Due to the presence of hydrophilic functional groups, they can imbibe large amounts of water and can be formulated using synthetic or natural polymers (biopolymers) [1]. One of the fascinating features of biopolymer-based hydrogels is their structural similarity with the extracellular matrix, apart from being biodegradable, biocompatible, abundant, and inexpensive [2], [3].

Carrageenan is a biopolymer that is extracted from rhodophytes. Depending on the sulfate content, carrageenan is classified as λ (lambda), κ (kappa), ι (iota), ν (nu), θ (theta), and μ (mu). Of all forms, κ-carrageenan is extensively adopted in clinical applications because it forms compact gels on its sulfate groups crosslinking with cations like K^+, Ca^{2+}, and Na^+ [4].

Chemical crosslinking is a versatile method used to improve the mechanical properties of biomaterials [5], [6]; however, chemical crosslinkers are often toxic. Instead, electrolytes like tender coconut water can be used as crosslinking agents! Tender coconut water has abundant potassium ions—approximately 250–300 mg of potassium is present in 100 g of tender coconut

water [7]. Therefore, using such natural alternatives ensures sufficient crosslinking and is less likely to be cytotoxic, cause inflammation, and are biodegradable and cost-effective.

Hydrogels are used extensively in tissue engineering applications [8]. Apart from being robust mechanically, an aspect of prime importance is that they must eliminate bacteria at the wound site to prevent infection. This can be achieved by loading them with substances that interfere with essential cellular processes or structures within the bacterial cell [9], [10].

Diclofenac sodium is a non-steroidal anti-inflammatory drug with a wide range of applications in clinical science due to its antipyretic and analgesic properties [11]; however, limited evidence exists on its antibacterial properties [12], [13], and its performance has never been compared with any well-known antibacterial substance. Diclofenac sodium is also hydrophilic and has low bioavailability [14] and one method to increase its hydrophilicity is to nanoemulsify it. Nanoemulsions are formed when two immiscible phases are exposed to a mechanical shearing force, segregating the dispersed phase into nano-sized droplets. Such particles possess great potential as a therapeutic vehicle because of their high hydrophilicity, drug-loading capacity, and remarkable ability to form bonds that protect their constituents from enzymatic degradation or hydrolysis [15] compared to their macromolecule form.

In this study, we designed κ-carrageenan hydrogels crosslinked with tender coconut water, KCl, and their combination. We analyzed their morphology, chemical bonding, compressive strength, water uptake capacity, degradation resistance, and cytotoxicity to assess their use as eco-friendly, natural, and cost-effective antibacterial biomaterials. The release behavior of the hydrogels was determined by loading nanoemulsified diclofenac sodium into them and analyzing their release at neutral pH. The antibacterial activity of the hydrogels loaded with diclofenac sodium was tested against *Staphylococcus aureus* and *Escherichia coli* and compared to those loaded with tetracycline: an antibiotic that shows antibacterial activity against a wide range of bacteria [16].

2. Materials and Methods

2.1. Materials

K-carrageenan was purchased from SNAP Natural and Alginate Products Pvt. Ltd. (Vellore, Chennai, India); potassium chloride (KCl), polysorbate-80, and dimethyl sulphoxide (DMSO) from Merck; and diclofenac sodium from Sigma Aldrich. Tender coconut water and castor oil were purchased from a local market in Manipal.

2.2. Preparation of κ-carrageenan hydrogels

K-carrageenan hydrogels were prepared using the microemulsion method [17], [18]. 1.5 g κ-carrageenan was added to varying amounts of polysorbate-80, and the solution was made up to 50 ml using deionized water (*Table 1*). The suspensions were stirred for 20 min at 600 rpm at 75 ℃. Castor oil was added to prevent foaming. The resulting solutions were poured onto 6-well cell culture plates (Himedia, India), allowed to stay for 24 h at room temperature, and removed.

Table 1. Chemical composition of the prepared κ-carrageenan hydrogels

Volume of polysorbate-80 (ml)	Volume of distilled water (ml)	Total volume (ml)
1	49	50
2	48	
3	47	
4	46	
5	45	
6	44	
7	43	
8	42	
9	41	
10	40	

2.3. Preparation of nanoemulsified diclofenac sodium-loaded crosslinked hydrogels

A nanoemulsion solution was prepared by mixing 1 mg diclofenac sodium in 1 ml polysorbate-80 and was introduced into a prepared κ-carrageenan suspension. Crosslinker solutions comprising sterilized tender coconut water, varying molarities of KCl, and their combination were prepared (*Table 2*) and crosslinked with the κ-carrageenan suspension using 6-well cell culture plates (Himedia, India). The obtained hydrogels were incubated in a hot air oven

(Servewell Instruments Pvt Ltd, India) for one hour at 60 ℃ and left for cooling at room temperature for 24 h. A control was maintained to compare the crosslinking potency.

Table 2. Chemical composition of the prepared crosslinker solutions

Molarities of KCl (M)	Volume of tender coconut water (ml)	Combination
5.0	25	25 ml 5.0 M KCl + 25 ml tender coconut water
4.5		25 ml 4.5 M KCl + 25 ml tender coconut water
4.0		25 ml 4.0 M KCl + 25 ml tender coconut water
3.5		25 ml 3.5 M KCl + 25 ml tender coconut water
3.0		25 ml 3.0 M KCl + 25 ml tender coconut water
2.5		25 ml 2.5 M KCl + 25 ml tender coconut water
2.0		25 ml 2.0 M KCl + 25 ml tender coconut water
1.5		25 ml 1.5 M KCl + 25 ml tender coconut water
1.0		25 ml 1.0 M KCl + 25 ml tender coconut water
0.5		25 ml 0.5 M KCl + 25 ml tender coconut water

2.4. Morphology analysis

The microstructure of the hydrogels was examined using EVO MA18 with an Oxford EDS (X-act) electron microscope. The samples were cut into 5 × 5 mm squares and vacuum-dried (Labline, Mumbai). The procedure was performed at 1,000x magnification with 10 kV energy after sputtering the samples with gold nanoparticles [19].

2.5. Chemical interaction analysis

The Fourier-transform infrared (FTIR) spectrophotometer (Shimadzu FTIR-8400) was used to comprehend the hydrogels' functional groups and bond linkages. The samples were dried and compressed into pellets using KBr in a ratio of 1:100 to perform the analysis [20].

2.6. Strength analysis

A universal testing machine (Shimadzu EZ-SX) was used to assess the elongation and compressive strength of the hydrogels. The hydrogels were split into 35 × 15 mm pieces, and the ends were held between two clamps and compressed at a pace of 1 mm/min under a load of 500 N [21]. Each test group contained three samples. The data received on load versus displacement was translated into nominal stress and nominal strain. Further, the stress-strain data was used to evaluate Young's modulus, compression strength, and energy. Young's modulus and energy values were obtained by curve fitting the linear zone and area. The compression strength corresponds to the highest nominal stress value.

2.7. Swelling studies

The water retention capabilities of the hydrogels were determined by weighing them at predetermined intervals after immersing them in deionized water at room temperature to calculate the amount of swelling that occurred.

The hydrogels' initial dry weight was recorded and then submerged in water for varying intervals. The inflated hydrogels were withdrawn from the water and pressed with blotting paper to remove any surface water, and their weights were recorded using an electronic balance. The percentage of swelling was calculated using *Equation 1* [22]:

$$\%\, Water\ retention\ = \frac{w_t\ -\ w_0}{w_0} \times\ 100 \qquad \mathbf{1}$$

where w_t is the weight of the hydrogels immersed in water at different intervals, and w_0 is the initial weight of the hydrogels.

2.8. Hydrogel degradation behavior

A phosphate buffer solution of pH 7.4 and temperature 37 °C was used for three weeks to determine the hydrogels' degradation rate. The initial weight of the hydrogels was recorded, and at a predetermined interval each day, one hydrogel was removed from the phosphate buffer solution, and its weight was recorded using an electronic balance after pressing with blotting paper [23]. The percentage of degradation that occurred in the hydrogels was calculated using *Equation 2*:

$$\%\, Degradation\ = \frac{w_0\ -\ w_t}{w_t} \times\ 100 \qquad \mathbf{2}$$

where w_0 is the initial weight of the hydrogels, and w_t is the weight of the hydrogels immersed in phosphate buffer solution at different intervals.

2.9. Cytotoxicity assessment

An MTT assay was used to determine the cell viability. 3T3 cells were grown in the hydrogels for 1, 3, and 5 days. 0.5 mg/ml MTT was added to the sterile hydrogels and incubated at 37 ℃ and 5% CO_2 for four hours. The formazan precipitate in the hydrogels was dissolved in DMSO, and the absorbance was measured at 570 nm using a multimode microplate reader (Perkin Elmer (Ensight) multimode plate reader HH34000000). The cell viability was determined by calculating the ratio of living cells to the total cells.

When exposed to the hydrogel environment, a live/dead viability kit was used to visualize the 3T3 cell viability (Thermo Fisher, U.S.A). The materials used were disinfected using UV irradiation prior to cell seeding. The wells were incubated for one hour at 37 ℃ with a calcein-AM stock solution and ethidium homodimer-1 stock solution. Calcein-AM, a cell-permeable dye, is transformed to green-fluorescent calcein by live cells, while ethidium homodimer-1 attaches to nucleic acids of cells with compromised membranes to create red fluorescence. A fluorescent microscope was used to capture images (Nikon Eclipse-TE2000-U).

2.10. Diclofenac sodium release behavior

The diclofenac sodium release behavior was analyzed using a UV-vis spectrometer (Shimadzu UV-1800). Diclofenac sodium-loaded hydrogels were immersed in a beaker containing 30 ml phosphate buffer solution buffer (7.4 pH). The beaker was kept in a shaking incubator (Labline Instruments, India) at room temperature, followed by taking 4 ml aliquots at predetermined intervals for 24 h. The sinking condition was maintained by adding 4 ml PBS to the beaker [24]. The cumulative diclofenac sodium release was calculated using *Equation 3* as described below:

$$Cumulative\ diclofenac\ sodium\ release\ (\%) = \frac{Cumulative\ amount\ of\ diclofenac\ sodium\ released}{Initial\ amount\ of\ diclofenac\ sodium\ added} \times 100 \quad (3)$$

2.11. Antibacterial assay

Diclofenac sodium-loaded hydrogels were sterilized under ultraviolet light for two hours before use. Freshly cultivated *Staphylococcus aureus* (*S. aureus*, ATCC 25923) and *Escherichia coli*

(*E. coli*, ATCC 25922) were uniformly distributed over Mueller Hinton agar, followed by placing the hydrogels and then made into 1 × 1 cm diameter pieces. The plates were incubated for 24 h at 37 °C in a bacterial incubator, and the inhibitory zones were measured.

2.12. Statistical analysis

All the experiments were carried out in triplicate, and the results were recorded as the arithmetic mean ± standard deviation. One-way analysis of variance (ANOVA) (GraphPad Prism 7 Software, La Jolla, USA) was used to perform the statistical analysis, and a P-value <0.05 was considered statistically significant.

3. Results and Discussion

3.1. Selection of κ-carrageenan hydrogel and crosslinker solution chemical composition

The sulfate groups in κ-carrageenan show an affinity for monovalent cations such as potassium and form a coil-to-helix conformational shift, followed by a helix aggregation that induces the growth of crosslinked polymeric networks [25]. We exploited this property to prepare κ-carrageenan-based hydrogels. We realized that when samples containing less than 6 ml polysorbate-80 were used to design κ-carrageenan hydrogels, they either remained semisolid without gelling or were difficult to remove, and when more than 6 ml polysorbate-80 was used, the solution was too thick to obtain an even distribution on the plate and in some cases, gelling started to take place in the solution beaker itself. Similarly, when less than 2 M KCl was used to crosslink the hydrogels, they were unstable, and when more than 2 M KCl was used, the hydrogels became excessively stiff and brittle. The sample strategy was also followed to determine the appropriate crosslinker combination concentration. Thus, 6 ml polysorbate-80, 2 M KCl, and 2 M KCl + tender coconut water were determined to be the best chemical compositions in their respective cases.

3.2. Physical and morphological properties

3.2.1. Morphology analysis

A scanning electron microscope (SEM) was used to study the surface topography of the hydrogels. The micrographs (*Figure 1*) were captured at 1,000x magnification and 10 kV accelerating voltage.

The SEM micrograph of κ-carrageenan hydrogels crosslinked with tender coconut water shows a cloudy surface compared to κ-carrageenan hydrogels with and without diclofenac sodium

(refer to online supplementary material *Figure S1*), indicating that crosslinking has occurred between κ-carrageenan and tender coconut water. When diclofenac sodium was added to the hydrogels, the surface morphology became slightly rough with the compound embedded into the matrix.

The SEM micrograph of κ-carrageenan hydrogels crosslinked with KCl shows their morphology to be drastically different from those crosslinked with tender coconut water—rough, irregular, and broad cracks! When diclofenac sodium was added to the hydrogels, the compound was trapped in the spaces within the cracks of the flaky κ-carrageenan matrix.

The SEM micrograph of κ-carrageenan hydrogels crosslinked with tender coconut water and KCl shows a smooth surface; however, it developed narrow cracks wherein diclofenac sodium was trapped when the compound was added to the hydrogels. Tender coconut water dissolved the potassium and chlorine ions, hindering the formation of their crystalline structures.

It can be inferred that crosslinking κ-carrageenan with tender coconut water results in a compact morphology, whereas crosslinking with KCl results in a morphology with broad cracks. The morphology of the hydrogels crosslinked with tender coconut water and KCl is preferred as it forms narrow cracks that facilitate efficient diffusion from the hydrogel matrix while maintaining structural integrity. These cracks significantly increase the surface area of the hydrogel exposed to the surrounding medium and provide additional pathways for molecules to diffuse out of the hydrogel.

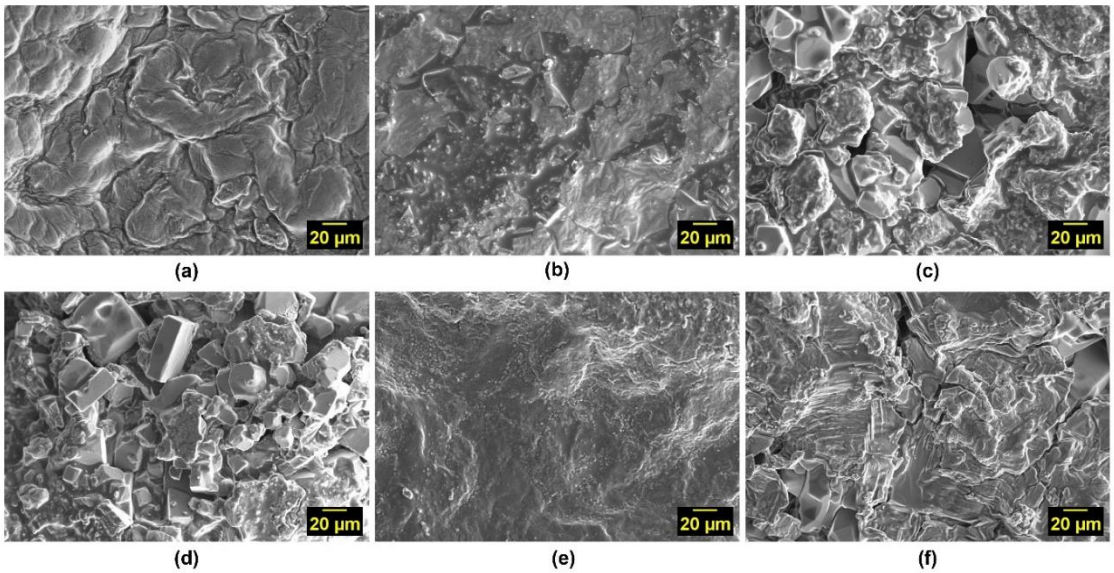


Figure 1. SEM micrographs of (a) κ-carrageenan + tender coconut water, (b) κ-carrageenan + tender coconut water + diclofenac sodium, (c) κ-carrageenan + KCl, (d) κ-carrageenan + KCl + diclofenac sodium, (e) κ-carrageenan + tender coconut water + KCl, and (f) κ-carrageenan + tender coconut water + KCl + diclofenac sodium hydrogels.

3.2.2. Chemical interaction analysis

FTIR is used to study the molecular interactions between different functional groups. *Figure 2* shows the FTIR spectrum of the various crosslinked hydrogels with/without diclofenac sodium. *Figure S2* depicts the FTIR spectra of κ-carrageenan, polysorbate-80, and diclofenac sodium. K-carrageenan shows characteristic peaks at 1260 cm^{-1} and 1048 cm^{-1} that indicate S=O stretching for sulfate, 921 cm^{-1} for anhydrous glycosidic linkage, and 845 cm^{-1} for galactose-4-sulfate [26]. Polysorbate-80 shows C−H stretching at 2919 cm^{-1} and 2878 cm^{-1}, C=O stretching at 1733 cm^{-1}, C−O−C stretching at 1109 cm^{-1}, and O−H stretching between 3700–3100 cm^{-1} [27]. Diclofenac sodium shows phenyl stretching between 700–800 cm^{-1}, C−N stretching between 1000–1400 cm^{-1}, C=C stretching at 1643 cm^{-1}, C=O stretching at 1737 cm^{-1}, C−H stretching between 2750–3000 cm^{-1}, C−Cl stretching at 711 cm^{-1}, and O−H stretching between 3250–3750 cm^{-1} [28].

In *Figure 2*, we observe a few bond shifts; however, new bonds are formed insignificantly. Therefore, it can be inferred that diclofenac sodium is inert with the κ-carrageenan hydrogel matrix when crosslinked with either tender coconut water, KCl, or their combination.

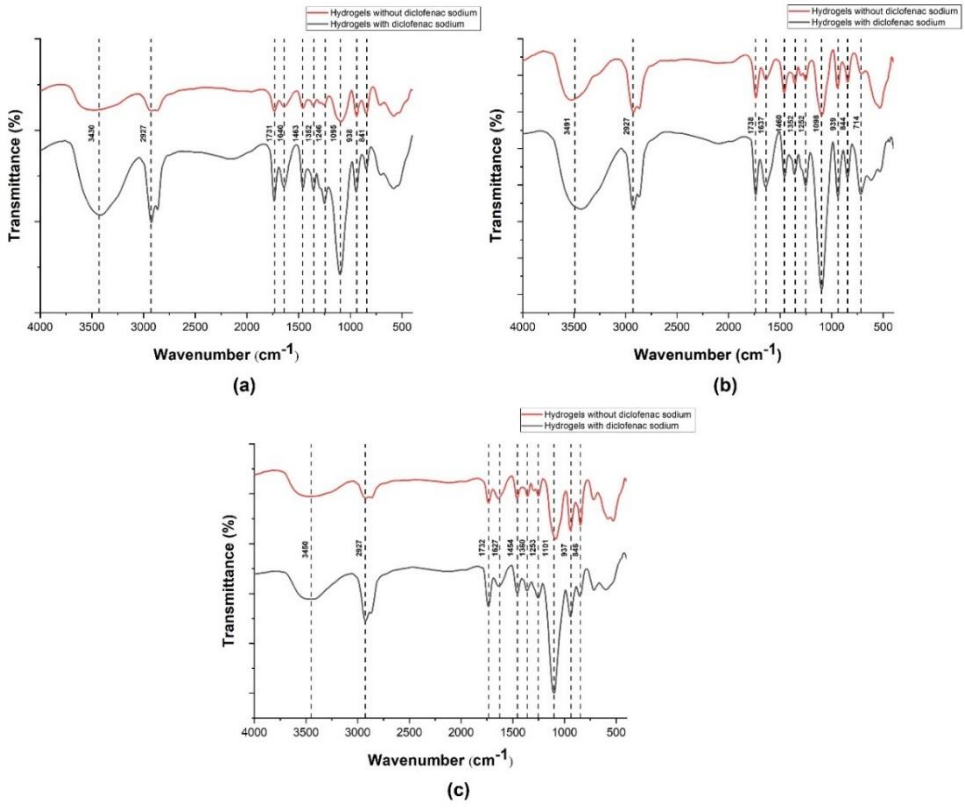


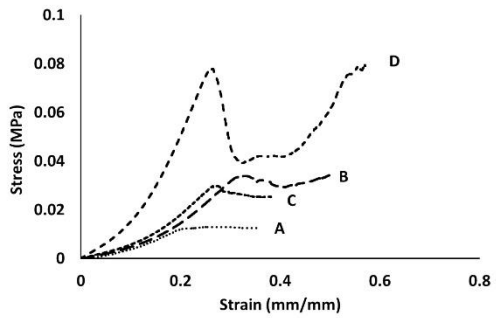
Figure 2. FTIR spectrum of κ-carrageenan hydrogels crosslinked using (a) coconut water with/without diclofenac sodium, (b) KCl with/without diclofenac sodium, and (c) coconut water and KCl with/without diclofenac sodium.

3.3. Mechanical properties

3.3.1. Physicomechanical analysis

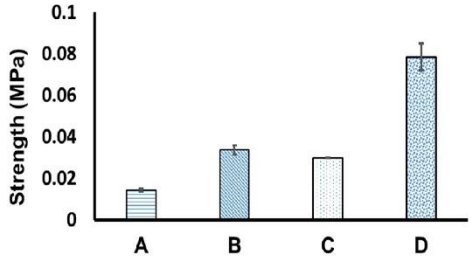
Figure 3 depicts the mechanical behavior of the uncrosslinked and crosslinked κ-carrageenan hydrogels as nominal stress-strain curves, which were used to determine Young's modulus (MPa), compressive strength (MPa), and energy (J)—characteristics that illustrate the impact of crosslinking.

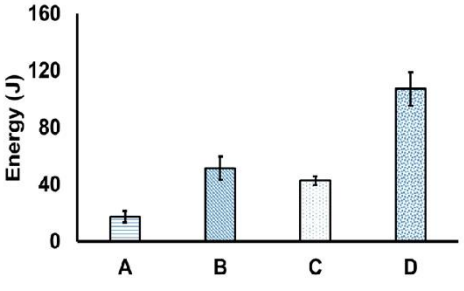
The free ions and dissolved electrolytes in crosslinkers alter the stress–strain relation, structural geometry, and types of bonds that form. From the stress–strain curve, it can be inferred that κ-carrageenan hydrogels crosslinked with tender coconut water and KCl possess the most compressive strength—an increase of 450% compared to the uncrosslinked hydrogels—and deformed the least when applying a high load. This is due to their synergistic crosslinking effect, as the combination contains high potassium ions that facilitate robust crosslinking. The hydrogels crosslinked with tender coconut water show the most elastic behavior compared to those crosslinked with KCl. Furthermore, tender coconut water contains much more than just potassium ions—proteins, enzymes, vitamins, minerals, sugars, antioxidants, and growth factors [29]—potentially resulting in greater strength through more interconnected crosslinks than KCl, which primarily provides ionic crosslinking. Thus, the combination of tender coconut water and KCl promotes crosslinking at a higher degree, rendering excellent strength due to potassium ions and bioactive components forming strong bonds with the κ-carrageenan matrix.



Hydrogel	Young's Modulus
K-carrageenan	0.0702 ± 0.006
Tender coconut water	0.1032 ± 0.003
KCl	0.0977 ± 0.006
Tender coconut water + KCl	0.3161 ± 0.018

(b)





A: K-carrageenan **B**: Tender coconut water **C**: KCl **D**: Tender coconut water + KCl

Figure 3. (a) Compressive behavior of the hydrogels, (b, c) Young's modulus and compressive strength of the hydrogels, and (d) Plot of energy absorbed by the hydrogels till the first peak in the load vs. displacement curve.

3.3.2. Swelling studies

Figure 4 shows the swelling behavior of the uncrosslinked κ-carrageenan hydrogels and those crosslinked with tender coconut water, KCl, and their combination. At equal intervals, the swollen weights of the hydrogels were recorded, which increased as time passed. However, after 12 h, the swelling rate decreased drastically, and the hydrogels reached saturation and exhibited negligible weight change. The weights were recorded until then and discontinued afterward, and the procedure was performed in triplicates.

The uncrosslinked and crosslinked hydrogels showed significant differences in their swelling behavior. All hydrogels displayed a controlled swelling pattern, and the uncrosslinked hydrogels were more porous than those crosslinked using tender coconut water, KCl, and their combination; thus, their water uptake capacity was superior to the others. Despite possessing good water uptake properties, their low mechanical strength would cause their matrix to disintegrate upon use in a saturated state. The hydrogels' structure became compact on crosslinking, holding relatively less water than uncrosslinked ones. The hydrogels crosslinked with KCl showed the least swelling due to the electrostatic repulsion within them that reduces

their swelling capacity and diameter [30]–[32]. Also, the broad cracks in their morphology decrease their ability to draw and hold water. The hydrogels crosslinked with tender coconut water display the most swelling among the crosslinked hydrogels; however, the hydrogels crosslinked with tender coconut water and KCl are the best as they display excellent swelling while possessing much greater strength than all other hydrogels (as discussed in **3.3.1**). These hydrogels retain water for longer without disintegrating and maintain good moisture levels.

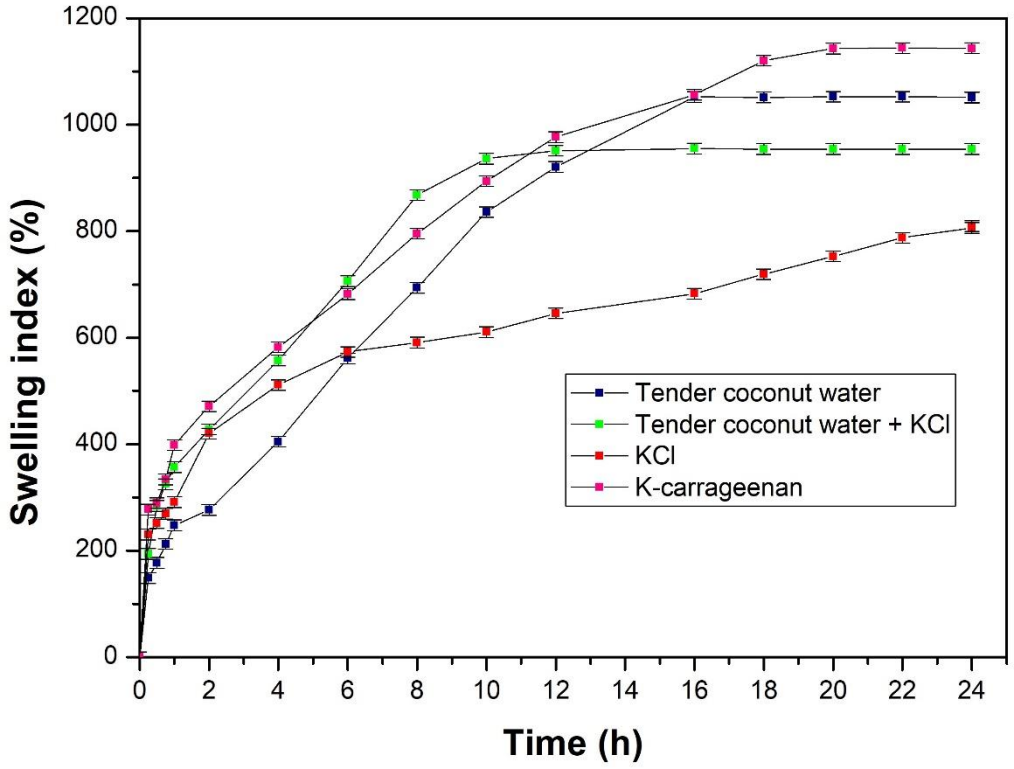


Figure 4. The swelling behavior of the uncrosslinked and crosslinked κ-carrageenan hydrogels.

3.3.3. Hydrogel degradation behavior

Figure 5 shows the degradation behavior of the uncrosslinked and crosslinked κ-carrageenan hydrogels plotted over time at pH 7.4. The uncrosslinked hydrogels degraded much faster than the crosslinked hydrogels due to the absence of crosslinking, resulting in poor strength, while those crosslinked with KCl showed a low initial degradation rate but later quickly degraded, possibly due to the cracks on their surface expanding over time. The hydrogels crosslinked with tender coconut water degraded at a rate similar to those crosslinked with KCl due to hydrolytic degradation caused by breaking down chemical bonds over time, primarily due to their high water uptake capacity [33]–[35]. Surprisingly, the hydrogels crosslinked with tender coconut water and KCl degraded much slower than all other hydrogels—just around 5% in over 20 days! This can be attributed to their superior mechanical strength (as discussed in

3.3.1.), and as they possess an excellent water uptake capacity (as discussed in **3.3.2.**), these can be used in applications such as wound healing to absorb exudates and omit the need for periodic replacement.

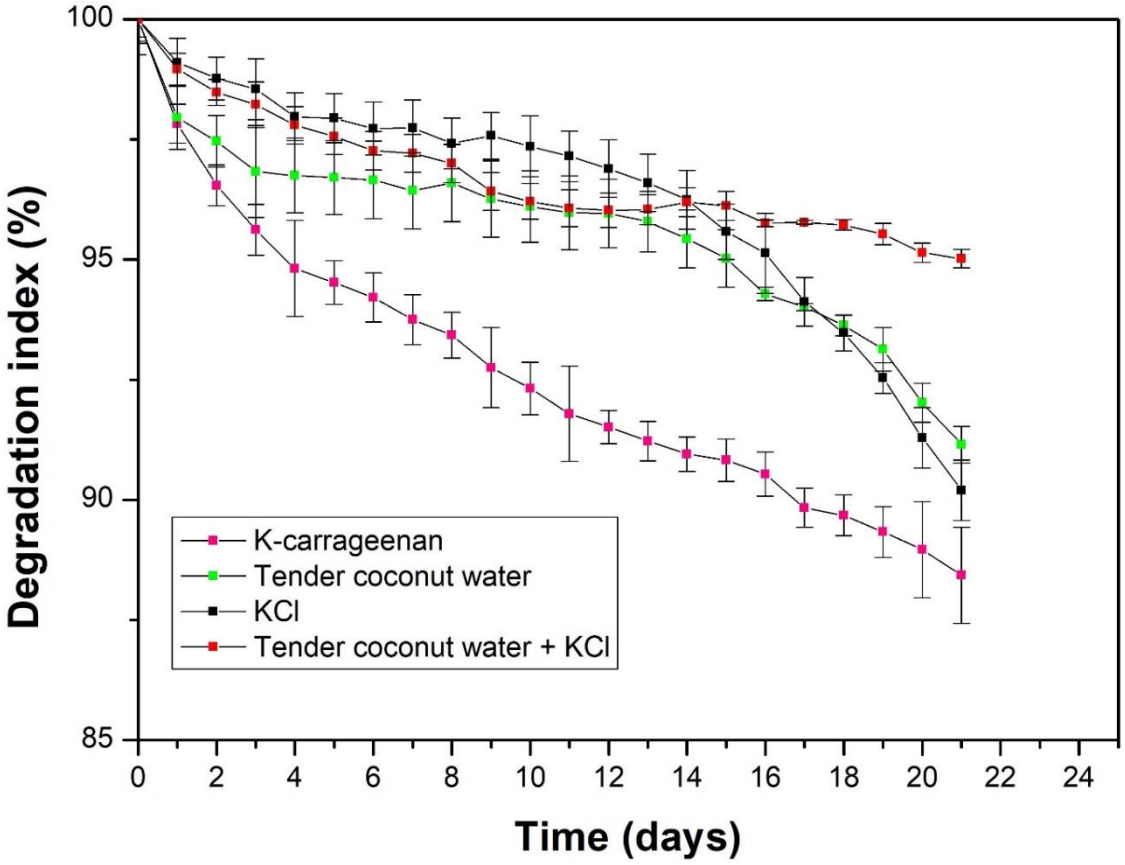
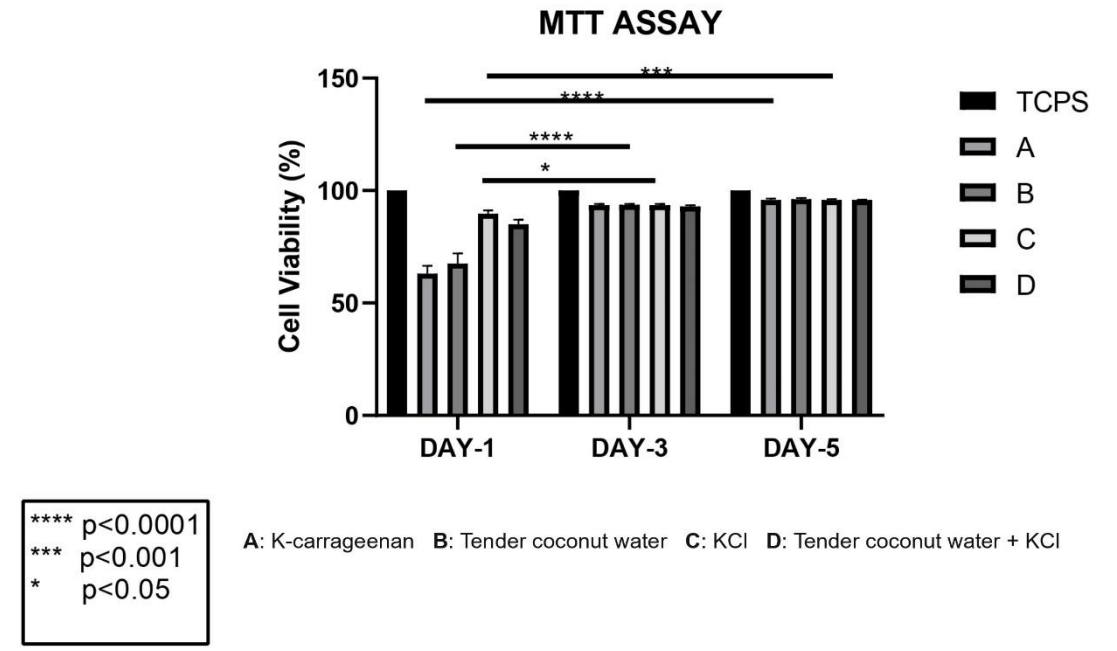


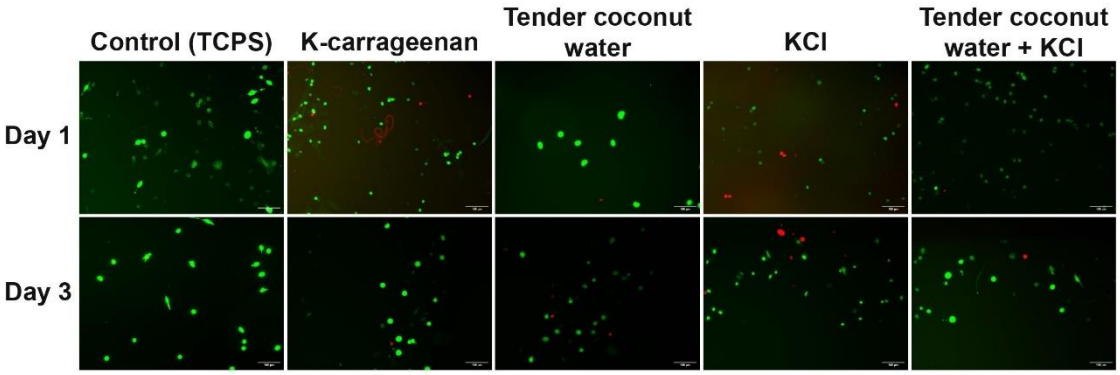
Figure 5. The in-vitro degradation behavior of the uncrosslinked and crosslinked κ-carrageenan hydrogels at pH 7.4.

3.4. Cytotoxicity assessment

Figure 6 shows the % cell viability of the uncrosslinked and crosslinked κ-carrageenan hydrogels after 24, 72, and 120 h, respectively. All hydrogels maintained nearly 100% cell viability on all test days, indicating insignificant cytotoxicity against 3T3 fibroblast cells. This shows that the uncrosslinked and crosslinked hydrogels are biocompatible and is further substantiated by the live/dead cell images taken one and three days after culture (*Figure 6*). Our results are within the acceptable limits according to ISO 10993-5-2009 [36].



(a)



(b)

Figure 6. (a) Graph showing 3T3 % cell viability of the uncrosslinked and crosslinked κ-carrageenan hydrogels. Each value is expressed as mean ± S.D., n = 3 independent experiments. (b) Live/dead images of 3T3 fibroblast cells cultured on the uncrosslinked and crosslinked κ-carrageenan hydrogels after one and three days.

3.5. *In-vitro* studies

3.5.1. Diclofenac sodium release behavior

Figure 7 shows the cumulative diclofenac sodium release from the uncrosslinked and crosslinked κ-carrageenan hydrogels plotted over time at pH 7.4. All the hydrogels displayed an initial burst release, followed by attaining a sustained release behavior over time. The uncrosslinked hydrogels released the most diclofenac sodium due to a drastic initial release caused by a lack of crosslinking. Out of the crosslinked hydrogels, the ones crosslinked with KCl released the most diclofenac sodium due to high diffusion through the broad cracks in

their morphology. In contrast, those crosslinked with tender coconut water showed a sustained release behavior due to their compact structure resulting from high crosslinking. However, the hydrogels crosslinked with tender coconut water and KCl showed a desirable release behavior as the initial burst release was the least among all hydrogels, and the release behavior was steady, gradual, and sustained. Therefore, using tender coconut water and KCl reduces the undesirable burst release from the κ-carrageenan hydrogels and helps maintain drug bioavailability over a long period.

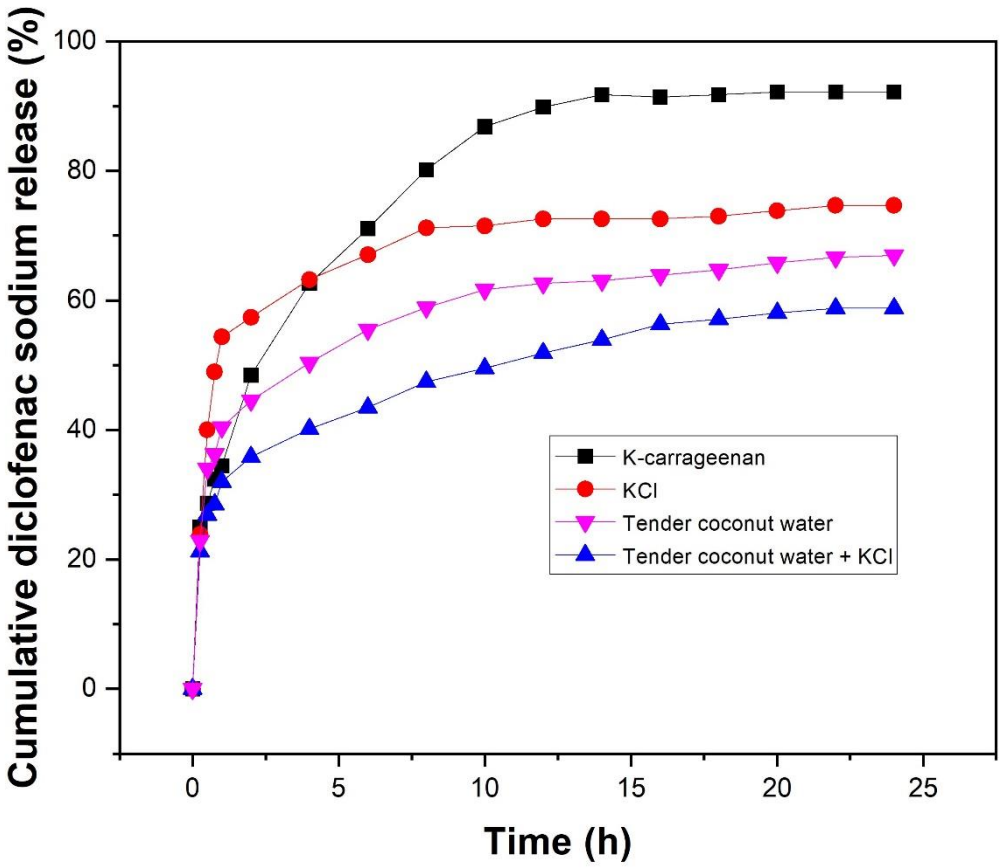


Figure 7. The in-vitro diclofenac sodium release profile from the uncrosslinked and crosslinked κ-carrageenan hydrogels at pH 7.4.

3.5.2. Antibacterial assay

Figure 8 shows Mueller Hinton agar disks containing the uncrosslinked and crosslinked κ-carrageenan hydrogels with and without diclofenac sodium, a uniform spread of *Staphylococcus aureus/Escherichia coli* over the agar disks, and the hydrogel's inhibitory zone. The 'Test' is a crosslinked hydrogel loaded with diclofenac sodium, whereas the '+ve Control' is a crosslinked hydrogel loaded with tetracycline.

The crosslinked hydrogels without diclofenac sodium showed an absence of an inhibition zone, which proves that the crosslinkers are inert against *Staphylococcus aureus* and *Escherichia*

coli. From the 'Test' samples, it can be inferred that all the crosslinked hydrogels were able to release diclofenac sodium into the bacteria spread and that diclofenac sodium is much more effective against *Escherichia coli* than *Staphylococcus aureus*—even more than tetracycline! The difference in the antibacterial effect is due to their resistance against diclofenac sodium, which we believe is due to being gram-positive or gram-negative.

The exact mechanism by which diclofenac sodium exerts its antibacterial effects is imprecise. Diclofenac sodium inhibited DNA synthesis in *Escherichia coli* (gram-negative) [12]; however, it is unclear whether it binds directly to DNA or degrades DNA by binding to RNA polymerase. Also, it moderately damaged the cell membrane when tested against *Listeria monocytogenes* (gram-positive) [13], and it was proposed that the compound has more than one mechanism of action that includes DNA synthesis inhibition and outer membrane damage. However, we disagree with the above notion as gram-negative bacteria possess lipopolysaccharides in their outer membrane, which act as a damage barrier to antibiotics [37] and are absent in gram-positive bacteria. If the damage caused to the outer membrane happened to be a mechanism of action, the antibacterial effect of diclofenac sodium would have been much more significant on *Staphylococcus aureus* than on *Escherichia coli*, as it would be more permeable in the former and can damage its outer membrane more significantly. Therefore, we propose that the antibacterial activity of diclofenac sodium relies on its ability to inhibit DNA synthesis and not on its ability to disrupt the outer membrane. Our analogy may require further research to prove its validity; however, we strongly believe in our critical analysis.

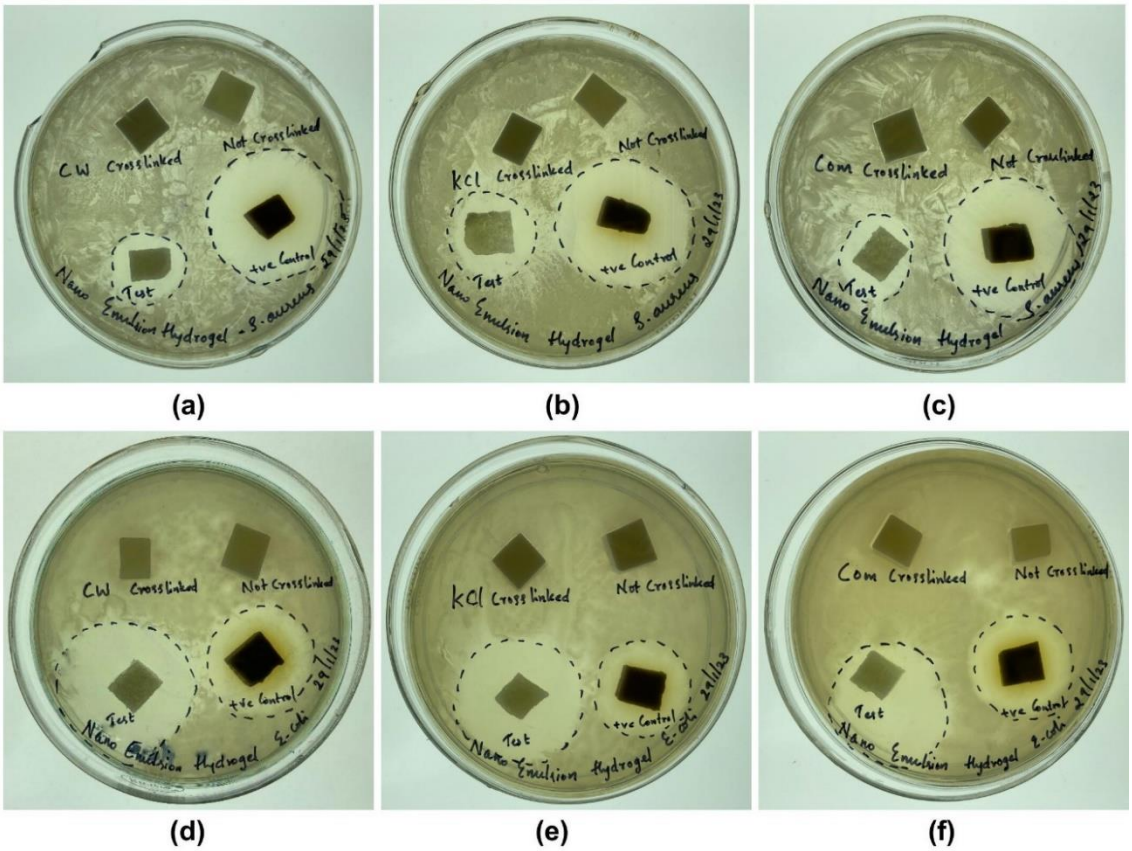


Figure 8. The zone of inhibition of (a–c) Staphylococcus aureus and (d–f) Escherichia coli exhibited by the uncrosslinked and crosslinked κ-carrageenan hydrogels. The 'Test' is a crosslinked hydrogel loaded with diclofenac sodium and the '+ve Control' is a crosslinked hydrogel loaded with tetracycline.

4. Conclusion and prospects

Our study aimed to investigate tender coconut water as a natural crosslinker alternative to KCl for κ-carrageenan by designing hydrogels crosslinked with tender coconut water, KCl, and their combination. The hydrogels were investigated for their morphology, chemical bonding, compressive strength, water uptake capacity, degradation resistance, cytotoxicity, and release behavior. The model drug chosen was diclofenac sodium, which is hydrophobic and thus had to be nanoemulsified to increase its hydrophilicity. SEM micrographs of the crosslinked hydrogels show that crosslinking with tender coconut water results in a compact morphology, whereas crosslinking with KCl results in a morphology with broad cracks. Interestingly, the crosslinker combination resulted in a morphology with narrow cracks, which promotes efficient diffusion from the hydrogel matrix. FTIR analysis confirmed that diclofenac sodium remained inert when introduced into the crosslinked hydrogel matrices, and the compressive strength analysis shows that the hydrogels crosslinked with tender coconut water and KCl had

much greater strength than others. Such hydrogels had an excellent water uptake capacity, were resistant to degradation, biocompatible, and released diclofenac sodium steadily and sustained for over a day. These results prove that the crosslinker combination is superior to KCl and can be used as an alternative to crosslink κ-carrageenan hydrogels. Also, the diclofenac sodium-loaded hydrogels showed a prominent antibacterial activity against *Escherichia coli*, and we suggest that diclofenac sodium exerts its antibacterial activity by relying on its ability to inhibit DNA synthesis and not on its ability to disrupt the outer membrane. However, our analogy may require more concrete evidence to prove its validity. It is an exciting prospect for the scientific community to explore, which would help establish the science behind our findings and persuade us to use diclofenac sodium against bacterial infections.

5. Conflicts of interest

The authors hold no conflicts of interest to declare.

6. Acknowledgments

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