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ORIGINAL RESEARCH PAPER

Plant-inspired adhesive and injectable natural hydrogels: in vitro and in vivo studies

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Abstract The development of alternative therapeutic treatments based on the use of medicinal and aromatic plants, such as *Juniper communis L.,* has aroused interest in the medical field to find new alternatives to conventional therapeutic treatments, which have shown problems related to bacterial resistance, high costs, or sustainability in their production. The present work describes the use of hydrogels based on sodium alginate and carboxymethyl cellulose, with combinations of juniperus leaves and berry extracts, in order to characterize their chemical characteristics, antibacterial activity, tissue adhesion test, cytotoxicity in the L929 cell line, and their effects on an in vivo model in mice to maximize the use of these materials in the healthcare field. Overall, an adequate antibacterial potential against *S. aureus, E. coli and P. vulgaris* was obtained with doses above 100 mg.mL^{-1} of hydrogels. Likewise, low cytotoxicity in hydrogels combined with extracts has been identified according to the IC_{50} value at 17.32 µg.mL^{-1}, compared to the higher cytotoxic activity expressed by the use of control hydrogels with a value at 11.05 µg.mL^{-1}. Moreover, in general, the observed adhesion was high to different tissues, showing its adequate capacity to be used in different tissue typologies. Furthermore, the invivo results have not shown erythema, edema, or other complications related to the use of the proposed hydrogels. These results suggest the feasibility of using these hydrogels in biomedical applications given the observed safety.

Keywords Biological activity · Bioadhesive injectable hydrogel · In-vivo skin irritation · *Juniperus communis* · Medicinal plants · Tissue engineering

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Introduction

The use of natural extracts (NE) has been part of the development of mankind throughout history in reason of their wide use in traditional medicine to recognize by means of empirical observation the positive effects of these in the evolution of certain diseases. In recently years, there has been a rising interest in traditional therapeutics in the pharmaceutical

industry, as well as in the flavoring of beverages and food, (Minero and Diaz 2017; Zheljazkov et al. 2017) through the use of NE. Among the great diversity of natural extracts, those from the Juniper (*Juniperus communis L.* (common juniper, Fig. 1) family have attracted attention due to their different antibacterial, anti-inflammatory, and antifungal properties, which make them suitable for applications such as traditional wound treatment, superficial treatment, and aroma therapy-based treatments. In addition, extracts from juniper species have shown anticancer effects due to their ability to inhibit abnormal cell proliferation (Lantto et al. 2009). Different compositional profiles have been detected depending on the part of the juniper plant from which the extract is obtained, such as the leaves, berries, or parts of the trunk (Radaukova et al. 2018). These variations in compositions obtained from different parts of the plant can have diverse effects on microbial activity, therapeutic properties, and their stability in matrices. It has been observed that juniper leaf extracts have an anti-inflammatory and antinociceptive effects on nephrotic tissue in mice models (Al-Attar et al. 2017). However, this type of model requires a more in-depth exploration of the role of juniper leaf extracts in preventing cellular stress and their effect on biological activity in tissues.

On the other hand, juniper berry-based extracts have been observed to influence cellular activity in aspects such as gene expression, cellular stress, and induction of neuroblast cell death (Raasmaja et al. 2019). Moreover, a significant reduction in the activity of adenomas and adenocarcinomas in animal models (Yaman et al. 2021) has been identified with the use of juniper berry extracts. The main reason for the properties of berries is related to the high content of α-pinene, which can modulate global gene expression and modify the metabolic action related to cancer cells in humans (Han and Parker 2017). Despite the extensive use of juniper berries since ancient times in culinary applications or traditional medicine, research on their activity is a topic that has aroused interest in order to increase their use in industrial production (Kacaniova et al. 2018). However, research in this area brings some challenges due to the compositional divergences among the different species of the juniper family. In addition, interest in analyzing the role of berry-based extracts has intensified in the last decade, by virtue of the anti-aging effects of these extracts, as they can prevent cellular oxidative stress in worms (Pandey et al. 2018).

However, the use of extracts is limited by their volatility and dispersion into the environment, leading to a loss of their properties over time when applied to tissues. Therefore, understanding the type of medium that can extend the properties of extracts and identifying the best types of extracts from berries or leaves is of great interest in the scientific community. In this sense, the use of this type of extract has involved experimenting with different vehicles for their transport, including encapsulated model matrices (Bajac et al. 2022), impregnation in polymeric fibers (Bigdarvishi et al. 2021; Tyliszczak et al. 2019), and polymer-ceramic composite materials (Zeng et al. 2014). Nevertheless, the different methods of transport and sources of extract present some challenges for their direct application on soft tissues such as skin, especially in the therapeutic treatment of cancer due to the sensitivity of the components. Therefore, a continuous study in this field is necessary to recognize the multiple possible uses of these extracts and their combinations.

This experimental study presents an overview of the functional characteristics, antibacterial activity,

Fig. 1 Images of *J. communis L.* plant

cytotoxicity, adhesion tissues response, and irritation evolution in in vivo models of polymeric hydrogels containing combinations of leaf and berry extracts from *J. communis L.* The aim is to enhance the current understanding of these compositions and their potential use in medicine as a cost effective, sustainable alternative biomaterial.

Experimental procedures

Materials and methods

Chemicals

Sodium alginate (SA, Mw: 4.2 × 105, M/G = 1.52), Carboxymethyl cellulose sodium (CM-cellulose; CMC-Na, Viscosity: 1000–1400 mpa.s, SD = 1.2), Glycerol (medical grade) and acetic acid, (CH_2COOH, glacial, ReagentPlus®, ≥ 99%) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Mineral water (Ca: 205.867 mg/L, Mg: 124.588 mg/L, K: 26.005 mg/L, Fe: 0.008 mg/L, Na: 138.181 mg/L, S: 176.11 mg/L, F: 0.64 mg/L, Si: 48.21 mg/L, Cl: 21.94 mg/L, CO_3^{-2}: 1433.5 mg/L) was obtained from Beypazarı Doğal Maden Suyu incorporated company in Türkiye. Ultrapure water was prepared by a Milli-Q50 SP Reagent Water System (Millipore Corporation, MA, USA) for the preparation of samples. Other reagents were of analytical grade.

Preparation of J. communis L. plant and extract

J. communis L. plant leaves and berries were collected above the village of İmaret, near Sivas, Türkiye, N 39 ° 41′ 54.3444″ E37° 1′ 5.6712″ at 1415 masl in 2022. The collected juniper leaves and berries were cleaned and spread out in a layer approximately 2 cm deep in a well-aerated shady place to dry for a month at room temperature before extraction. The extraction was carried out using Soxhlet extractors in accordance with TAPPI standards (TAPPI 1988). The subsamples for the extraction consisted of 50 g air-dried leaves and berries, which were ground into fine powder immediately prior to distillation. The biomass-to-solvent ratio was 1:10. The grounded plant material was transferred to an extraction thimble which was placed in a Soxhlet apparatus with a connected reflux condenser. The appropriate solvent (ethanol and ethyl acetate) was then added to the flask and used as a solvent. After 6 h of extraction and complete exhaustion of the *J. communis* herbal material, the obtained extracts were evaporated to dryness under vacuum at 40 °C and stored in sterile glass bottles at 4 °C until further experiments. In this study, a concentration of 5% w/v of leaf and berry extracts in hydrogels was used due to several factors. Firstly, higher concentrations of extract in the hydrogel may result in precipitation or aggregation of extract components, which could negatively impact the physical properties of the hydrogel. Secondly, the concentration range of the extract plays a crucial role in determining the in vivo activity of the hydrogels because it contains specific bioactive compounds, or there may be synergistic effects between different components. Finally, from an economic point of view, the cost of the extracts and their availability may also have been a limiting factor in the concentration used. Using a lower concentration may have made the study more economically feasible while still yielding valuable results.

Preparation and pharmaceutical compositions of sodium alginate/sodium-carboxymethyl cellulose (SA/CMC-Na) hydrogel

The hydrogel solution was prepared as follows: a homogeneous solution of mineral water, which offers a stable host for various ions and provides high ionic conductivity, stability, and viscosity was obtained by adding 0.5% v/v of CH_2COOH in 100 ml at RT under vigorous stirring (pH ~ 4.8 to 5) (Li et al. 2022). Next, 1.5% w/v CMC-Na and 1.5% w/v SA powder were slowly added and stirred at 37 °C until complete solubilization. To complete the carboxymethylation reaction, the reagents were mixed together by slowly adding 6.5% v/v glycerol to form a uniform sticky solution at 37 °C for 6 h in a water bath. To prepare SA/CMC hydrogels loaded with *J.communis* plant leaves extract (designated as JL/SA-CMC) and berries extract (designated as JB/SA-CMC), leaf and berry extracts (5%w/v) were respectively added to the SA/CMC mixture to obtain a final composition of the SA/CMC hydrogel. One set of 10 cc syringes was filled with the SA/CMC hydrogel, and another set of 10 cc syringes was filled with JL/SA-CMC and JB/SA-CMC hydrogels. The syringes were capped with a male Luer cap. The gel-filled syringes were

then placed into a Tyvek pouch and sterilized in an autoclave at 110 °C for 30 min. The hydrogels were allowed to cool at room temperature (22 °C ± 2 °C) under an aseptic environment to achieve the desired hydrogels.

Fourier transform infrared spectroscopy (FTIR) analysis

The chemical structure of SA/CMC, JL/SA-CMC and JB/SA-CMC hydrogels was characterized by infrared (IR) spectroscopy. To evaluate the functional groups of the hydrogels, a sample of the 20 mg hydrogels was dried at 45 °C for 12 h. The spectra were measured using a Fourier transform (FTIR) spectrophotometer (Bruker Alpha II spectrometer, Germany) in the wavelength range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1}.

Antimicrobial activity

The antimicrobial activity of the hydrogels was quantitatively evaluated using the broth micro-dilution method according to the Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI 2020). Three indicator microorganisms, including *Staphylococcus aureus* ATCC 292123, *Escherichia coli* ATCC 25922, and *Proteus vulgaris* ATCC 7829 were used in the antimicrobial activity study. The stock solution of the hydrogels was prepared by dissolving 1 g of hydrogel in 2.5 ml of sterile dH_2O and sterilized by autoclaving at 110 °C for 5 min. Dilutions were then prepared in various concentrations ranging from 300 $mg.mL^{-1}$ to 25 $mg.mL^{-1}$ in Mueller Hinton Broth (MHB) by taking a sample from the stock solution. The study was carried out as follows: after 18 ± 2 h incubation at 37 °C in MHB medium, the densities of the indicator microorganisms were adjusted to McFarland standard No: 0.5 using the same medium. Firstly, dilution series of samples (100 µl) were transferred to microtiter plates and then adjusted indicator microorganism (100 µl) was added to the wells. Ciprofloxacin, indicator microorganism suspension, and sterile MHB was used as the reference antimicrobial agents, negative control, and positive control, respectively. All assays were performed in triplicate. After all, microtiter plates were incubated at 37 °C for 24 h, the absorbance was recorded at 600 nm on a microplate reader (Spectro Star Nano, Germany) and the lowest concentration that inhibits the indicator microorganism growth was determined as the minimum inhibitory concentration (MIC) value. Additionally, to evaluate the antibactericidal effect of sub-MIC concentrations, the inhibition ratio (%) was calculated using Eq. 1 (Kamoun et al. 2015):

$$\boldsymbol{IR}(\%) = 100 - \left[\frac{OD_{GS} - OD_{B1}}{OD_{NC} - OD_{PC}}\right] \times 100 \quad (1)$$

where; the OD_{B1} is the absorbance of medium containing only dilutions of the hydrogel samples, OD_{GS} is the absorbance of the bacterial solutions after incubating growth medium, OD_{NC} is the absorbance of the negative control and OD_{PC} is the absorbance of the positive control.

In vitro cytotoxicity assays

Mouse fibroblast L929 cells were used for the cytotoxicity assays of the hydrogels. The cells were grown in a medium composed of DMEM (Gibco, Grand Island, NY, USA), % 10 FBS (Capricorn, USA), % 1 Penicillin–Streptomycin (100 IU/mL). For the study, 1 ml of each hydrogel (SA/CMC, JL/SA-CMC and JB/SA-CMC) was diluted in 5 ml DMEM, vortexed for 5 min, and incubated for 24 h. The cells were seeded on 96-well plates (100 µL/well), and then exposed to dilutions of the hydrogels. From each hydrogel dilution, different volumes were extracted to achieve different concentrations of the hydrogels in the cell growth medium (100 $\mu g.mL^{-1}$, 50 $\mu g.mL^{-1}$, 25 $\mu g.mL^{-1}$, 12. 5 $\mu g.mL^{-1}$, 6.25 $\mu g.mL^{-1}$, 3.125 $\mu g.mL^{-1}$, 1.56 $\mu g.mL^{-1}$, and 0.78 $\mu g.mL^{-1}$). The cells were then incubated at % 5 CO_2 atmosphere and 37 °C for 2 days. Cytotoxicity effects on L929 cell lines were determined by the XTT assay 24 h after exposure to the different concentrations. Cell viability was measured in a microplate reader in the reference range of 475 nm. It was determined according to the observed orange color intensity at the end of the incubation period. All absorbances were compared with control samples (cells without any test concentration) representing %100 cell viability. The data were presented graphically, and the representative IC_{50} for each of the hydrogels was estimated by regression. All processes were carried out in accordance with the ISO 10993-5 protocol.

Primary skin irritation test

The evaluation of skin irritation is a major index of dermal safety in pharmaceutical/cosmetic applications. The skin compatibility of the hydrogels was conducted using the primary skin irritation test, which can be considered as the first clinical step in the safety evolution. The study was carried out after the review and approval of the research project by the Research Ethics Committee of Sivas Cumhuriyet University with approval number: 65202830–050.04.04–681. Dermal safety was conducted according to ISO-10993-10:2021 (Tests for irritation and skin sensitization) while taking into account the procedures followed in previous works in the literature (Horng-Huey et al. 2013; Jibry and Murdan 2004). Briefly, six male Albino mice weighing approximately 20 g (5 weeks old) were obtained from the animal hospital of Sivas Cumhuriyet University, Sivas, Türkiye. The mice were caged in a laboratory with standardized environmental conditions (20 °C ± 2, % 35–45 Relative humidity) and a constant day/night cycle. They were fed with a standard rat chow diet and water ad libitum in a specific pathogen-free laboratory for one week. 24 h prior to the test, the hair from the back of each mouse was cleaned-shaved on both sides of the dorsal skin to expose adequately large test areas. The dorsal skin of the mice was divided in four test sites (approximately 1.5 cm × 1.5 cm) for application and observation. The hairless dorsal skin was wiped with EtOH. The untreated control side was marked out on their upper backs. All mice were topically treated with their respective formulations once daily on day 1 of the 3-day study, with an application time of 24 h, 48 h, and 72 h. 50 mg of SA/CMC, JL/SA-CMC and JB/SA-CMC hydrogel was applied onto the test area on the lower/upper back and carefully rubbed in. At the end of the each application time, the treated area was gently wiped with water-soaked gauze to remove any residual vehicle from the skin surface.

For the dermatologically correct assessment of differences in skin surfaces, each of the mice was kept in separate cages for the duration of the experiment. Moreover, all the visual assessments and the clinical evaluation were performed by a veterinary surgeon (not the investigator who applied the preparations). The skin irritation of hydrogel samples, such as erythema or edema, was evaluated by the scoring system of Draize dermal irritation test, including (0): no erythema or no edema, (1): very slight erythema or edema, (2): well-defined erythema or edema, (3): moderate erythema or edema, (4): severe erythema or edema.

Tissue adhesion of the hydrogels

Strong tissue adhesion is essential for in situ hydrogels used in tissue bonding to resist mechanical forces during dynamic movements. Additionally, adhesive hydrogels must be capable of readily adhering to a variety of fresh living tissues, including pulmonary, gastric, spleen, cutis, muscle, adipocyte, and femur (Xiaoxuan et al. 2020). To demonstrate this in an experimental study, 100 mg of the freshly prepared hydrogel was applied to one side of the tissue of the mice. Then, the surface was lightly pressed manually, and the pressing surface was covered with latex gloves to avoid interfering with the interaction of the hydrogels and the tissue. The adhesion was evaluated for 10 minutes against gravity, after which the holding time was considered finished, concluding the test. In order to vividly display the adhesive behavior of the prepared hydrogels with various biological tissues, the composition of the matrix hydrogel and the extracted combinations with the matrix hydrogel were evaluated on each type of tissue.

Data analysis

All experimental results were analyzed using Origin Pro 2020 (Origin Lab, Northampton, MA, USA), Image Tool software and Sigma Plot for Windows Version 12.3 (Sigma Plot Software, Palo Alto, CA, USA). The data were expressed as mean ± standard deviation (SD) of the indicated number of experiments. Statistical analysis was performed using ANOVA followed by Student's t-test ($*p < 0.05$) for independent variables. The erythema/edema scores were evaluated using the Draize dermal irritation scoring system (DDISS). For all tests, a P value < 0.05 was considered statistically significant.

Results and discussion

Fourier transform infrared spectroscopy (FTIR)

Figure 2 shows the FTIR spectra obtained for pure SA/CMC, JL/SA-CMC and JB/SA-CMC hydrogels in the wavenumber range from 500 cm^{-1} to 4000 cm^{-1}.

Stretching vibrations of the O–H bonds are observed at 3270 cm^{-1} in all cases. In addition, the bands at 2933 cm^{-1} and 2884 cm^{-1} show symmetric and asymmetric vibrations of aliphatic chains of sodium alginate and carboxymethyl cellulose. The peak at 1599 cm^{-1} is associated with the vibrations of the carbonyl group (–C=O) in alginate. The bands at 1411 cm^{-1} and 1027 cm^{-1} are considered characteristic vibrations of –COO and –C–O bonds in alginate and carboxymethyl cellulose. The bands at 1250 cm^{-1} and 1213 cm^{-1} are associated with the vibration of –CO groups (Deepa et al. 2016; Huq et al. 2012). Moreover, the characteristic bands of carboxymethyl cellulose are observed at 1107 cm^{-1}, which is related to vibrations of the C–OH groups (Habibi 2014). The peak at 1323 cm^{-1} is associated with stretching vibrations of the COO functional group in sodium alginate (Lan et al. 2018). In addition, the associated bands between 993 and 607 cm^{-1} are considered characteristic of alginates due to their structure dominated by pyranose rings, which usually present vibrational bands in this region (Larosa et al. 2018). It is noted that in this region, the signals around 993 cm^{-1} are linked to deformation vibrations of C_3–O_3–H_3 bonds (Hou et al. 2018). At 921 cm^{-1}, the deformation vibrations of C–C–H bonds are associated, while at 850 cm^{-1}, the deformation vibrations of C–O–C (glycosidic linkage) are observed. At 817 cm^{-1}, the deformation vibrations of the ring in the C–C–O groups are related. Lastly, the signals at 597 cm^{-1} are related to ring breathing vibrations (Cardenas-Jiron et al. 2011). Nonetheless, with the incorporation of the juniper extracts, a shift to 599 cm^{-1} is observed, as well as a peak with lower intensity than in the matrix hydrogel. The shift is explained by an interaction of the terpene groups of the extracts, which slightly modify the electronegative activity of the carbon bonds of the alginate in the matrix hydrogel.

Evaluation of antimicrobial activity of hydrogels

The MIC values of hydrogel compositions against indicator microorganisms are shown in the Table 1. It is observed that the application of the hydrogels can provide an adequate degree of bacterial inhibition, with the minimum antibacterial activity observed at concentrations of 100 $mg.mL^{-1}$ against the inhibition of *S. aureus*. It was not observed that the incorporation of extracts of leaves and berries changes the MIC value of the prepared hydrogel. It is identified that in the case of *E. coli*, the MIC value was detected as 150 $mg.mL^{-1}$, and the incorporation of the extract did not modify this value. In the case of inhibition against

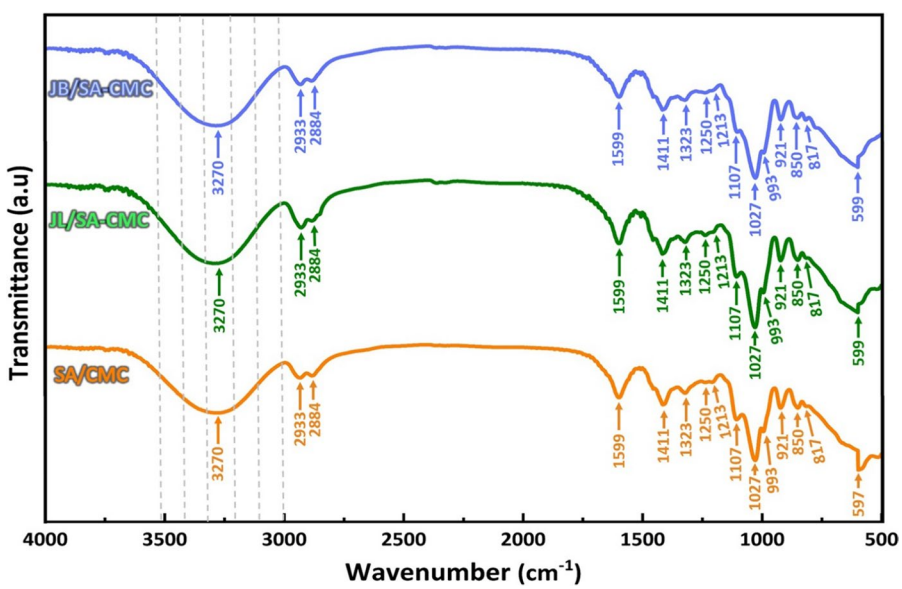

Fig. 2 FTIR spectra of hydrogels

Table 1 Minimum Inhibition Concentrations (MIC, $mg.mL^{-1}$) of different hydrogel compositions against indicator microorganisms

Compositions	Minimum inhibition concentrations ($mg.mL^{-1}$)		
	S. aureus	*E. coli*	*P. vulgaris*
SA/CMC	100	150	150
JL/SA-CMC	100	150	100
JB/SA-CMC	100	150	150
Ciprofloxacin ($C_{17}H_{18}FN_3O_3$)	< 0.0019	< 0.0019	< 0.0019

P. vulgaris, the MIC value was detected as 150 mg. mL^{-1} for the hydrogel without extract, but with the incorporation of leaf extract at 150 $mg.mL^{-1}$, this value was decreased to 100 $mg.mL^{-1}$. In the case of the combination with berry extracts, it was observed that the MIC value did not change.

When evaluating the antibacterial effect of sub-MIC concentrations, a difference in antimicrobial activity was observed between matrix and extract-added compositions. The antimicrobial activity of the matrix and hydrogel with juniper leaf extract against *E. coli* was completely lost at a concentration of 100 $mg.mL^{-1}$. In contrast, in the hydrogel with juniper berries extract, at the same concentration, % 73.9 ± 3.1 bacterial inhibition was observed. Similarly, for *P. vulgaris*, % 25.8 ± 4.5 bacterial inhibition was observed in the matrix at 100 $mg.mL^{-1}$ concentration, while % 90 ± 3.2 bacterial inhibition was observed in the hydrogel with juniper berries extract. These results support that the antimicrobial activity changes according to the concentration used.

Pathogenic microorganisms that colonize the skin surface, such as *S. aureus*, can cause atopic dermatitis, leading to a significant reduction in the quality of life. In addition, some pathogenic microorganisms can cause serious infections when they penetrate the skin barrier and proliferate in the cells. In this context, it is important to apply these hydrogels to the skin surface to provide an alternative to the use of drugs, as the current treatment can lead to bacterial resistance.

The present study has also observed the antibacterial activity of combinations of carboxymethyl cellulose and sodium alginate-based hydrogels. Despite previous studies observing low antimicrobial activity (Asadpoor et al. 2021; Kumar et al. 2019; Puscalescu et al. 2020), of the components separately, their combination can increase their antimicrobial activity (Han and Wang 2017; Shao et al. 2015). The antimicrobial activity of sodium alginate and carboxymethyl cellulose combinations is based on different parameters such as the amount used, other components in the matrix, pH of the medium, and temperature of incubation (Abdellatif Soliman et al. 2021; Han and Wang 2017). Therefore, in this study, the presence of antimicrobial activity in the prepared hydrogel matrix is mainly attributed to its intrinsic composition. Previously, antimicrobial activity was generally determined using the agar disc diffusion method (Han and Wang 2017; Kamoun et al. 2015; Kumar et al. 2019). However, this methodology has disadvantages in evaluating the antimicrobial activity of hydrogels or aqueous compounds due to interactions of the components and their viscosity, which may interfere with bacterial growth or create certain in homogeneities in the dispersion. This study provides insight into the antimicrobial activity that can result from hydrogels based on sodium alginate and carboxymethyl cellulose and their combinations with juniper extracts, demonstrating their potential use as bacteriostatic agents in dermal treatments as an alternative to high value drugs and ointments.

In vitro cytotoxicity evolution of hydrogels

Figure 3 shows the dose–response curves of different hydrogels combinations. Based on the regression of the points on this curve, the value of half maximal inhibitory concentration (IC_{50}) for the cell development of the fibroblast cells of L929 mice has been established. An IC_{50} value of 11.05 $\mu g.mL^{-1}$ was calculated for the hydrogel matrix, 15.19 $\mu g.mL^{-1}$ for the combination with juniper leaf extracts, and 17.32 $\mu g.mL^{-1}$ for the hydrogel containing berry extracts. In cosmetology, tissue exposure is usually measured in micrograms per volume of hydrogel, emulsion or ointment. In this sense, the IC_{50} provides a first overview of the possible toxic effects at the microscopic level on cell development. Therefore, a product with a low IC_{50} has a higher probability of promoting cellular toxicity. Incorporating extracts into hydrogels has been found to increase cell tolerance to combinations of alginate and carboxymethyl cellulose matrices. It has been observed that hydrogels consisting of

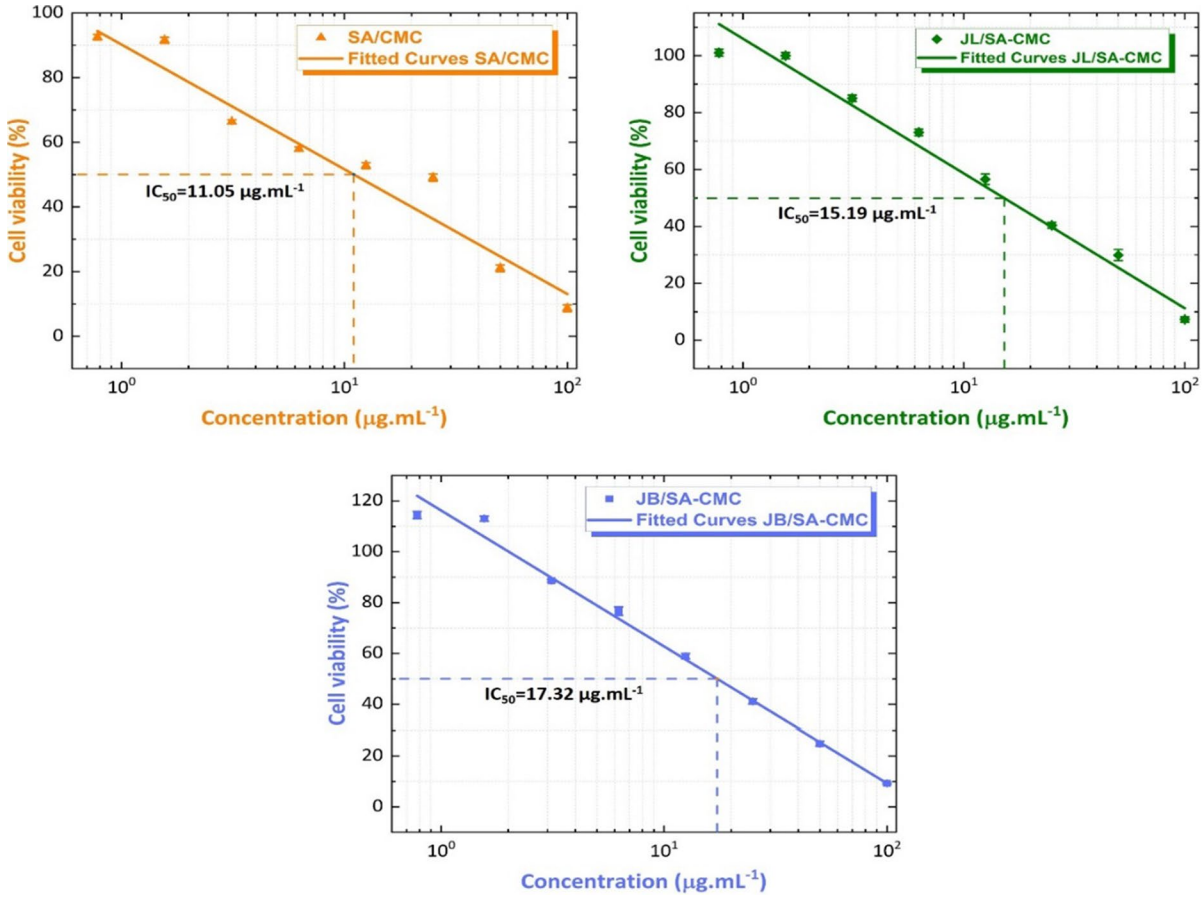

Fig. 3 In vitro cyctoxicity assay results to determine the IC_{50} value of the different hydrogels and analyze their effect on cell viability (%)

sodium alginate and carboxymethyl cellulose exhibit different behaviors with respect to cell viability, as alginates generally have poor characteristics related to cell development (Hurtada et al. 2022). Therefore, combining these components with other substances can improve their ability to enhance cell development, such as the combination with carboxymethyl cellulose. Studies have shown that carboxymethyl cellulose promotes the stability of the cell medium, leading to increased cell viability over time (Jeong et al. 2018).

In the case of juniper leaf extracts, positive effects have been observed in preventing cell deterioration. This due to the fact that extracts from juniper species usually have a low tendency to cause cell degradation, making them a feasible candidate for therapeutic treatments, especially in preventing damage to healthy tissues during chemotherapy (Och et al. 2015). When combined with polymeric matrices as a medium of transport, the incorporation of juniper extracts has shown beneficial effects. The observed behavior is related to the high content of polyphenols in juniper berry extracts, which increases antioxidant activity and protects against cell deterioration (Ivanova et al. 2018). This implies that to promote cell deterioration at a macroscopic level, high concentrations of the extract are necessary, especially in the treatment of cancer cells (Ivanova et al. 2021).

Primary skin irritation test

The in vivo safety of the prepared hydrogels with respect to skin irritation was tested in mice (Fig. 4). The skin sites of where the hydrogels were applied on all mice were visually assessed at 24, 48, and 72 h after the removal of the hydrogels, as shown in Fig. 5.

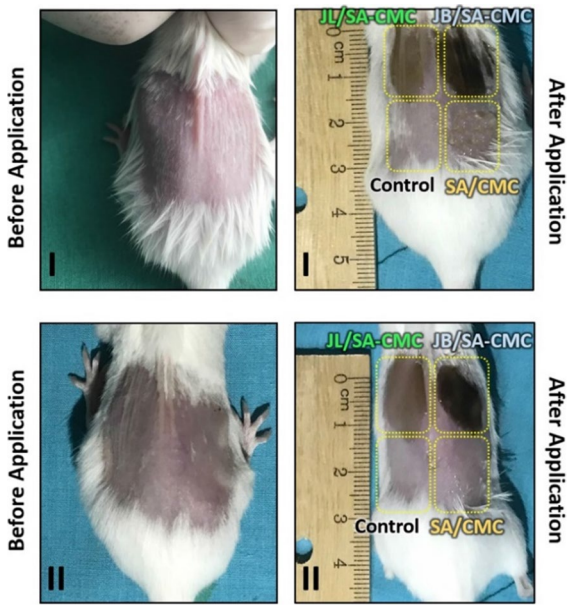

Fig. 4 Photographs of primary skin irritation in vivo studies

It was observed that after the study period there was no skin irritation or inflammation, and according to the ISO-10993–10:2021 classification, an indication of "0" was presented due to the absence of edema or erythema after evaluation for 24, 48 and 72 h (Fig. 5). Additionally, no abnormal capillary growth factors were observed during this period, suggesting that the use of this type of hydrogels does not generate significant alterations in the development of epithelial tissue during its use. The observed results may be related to the high antioxidant capacity offered by juniper extracts (Al-Attar et al. 2017; Pandey et al. 2018). Despite knowledge of their antioxidant activity, the possible effects of their use based on polymeric vehicles such as those used in this study were not previously known on epithelial tissue.

Tissue adhesion test

Juniperus is a medical plant that contains several bioactive compounds, including terpenes, flavonoids and phenolic acids. Adhesive hydrogels containing extracts of Juniperus leaves and berries may have several potential applications in tissue adhesion and repair in various organs, which is the reason this property has been evaluated in hydrogels. For instance, in the case of the using adhesive hydrogels in lung tissue, the hydrogel could be used in the treatment of lung infections to prevent the formation of bacterial biofilms (Klančnik et al. 2018). In the case of gastric tissue, future applications could be in the treatment of acute gastric ulcers or mixed with dietary supplements, providing a view of the adhesion capability of these hydrogels on the gastric tissue (Kesbiç 2019). On the other hand, the use of hydrogels based on a mixture of juniper extracts could be used as a vehicle for pesticides (Semerdjieva et al. 2021). Hence, the identification of their adhesive properties on cutis tissue provides knowledge about the advantages and disadvantages that this type of materials has on the cutis of animals. In each case, the specific application and method of use of adhesive hydrogels containing Juniperus extracts will depend on the particular condition and the properties of the hydrogel used. Tissue adhesion is a major factor in the practical application of tissue adhesives, wound dressings/patches, and wearable medical devices (Ghobril et al. 2015; Qu et al. 2018). The adhesion properties of produced hydrogels with various biological tissues were investigated, and the results are shown in Fig. 6. Biological tissues derived from mice, including the pulmonary, gastric, spleen, cutis and muscle, could easily adhere to the SA/CMC, JL/SA-CMC, and JB/SA-CMC hydrogels. Adequate adhesion of the hydrogels to the biological tissues was observed in all cases, despite the incorporation of the extracts; no significant changes were observed in their adhesive behavior. The observed performance may be related to the functional groups that compose the hydrogel, as well as the interactions between the tissues. It has been observed that the adhesion of hydrogels to tissues is related to the interactions established between the carboxylic groups of the hydrogel matrices and the interactions with amide formation, which they usually cover part of the biological material. This adhesion is usually mediated by the formation of covalent bonds between the carboxyl-amide groups (Sun et al. 2021). This suggests that the use of the present matrix is feasible for its extensive use on a variety of tissues as it guarantees the adherence and interaction of the components during the treatment. Likewise, one of the factors that provides a positive value to adherence is that it prevents changes or disruptions during the treatment that could affect the final activity as well as the biological effects that this type of hydrogels offers on tissues.

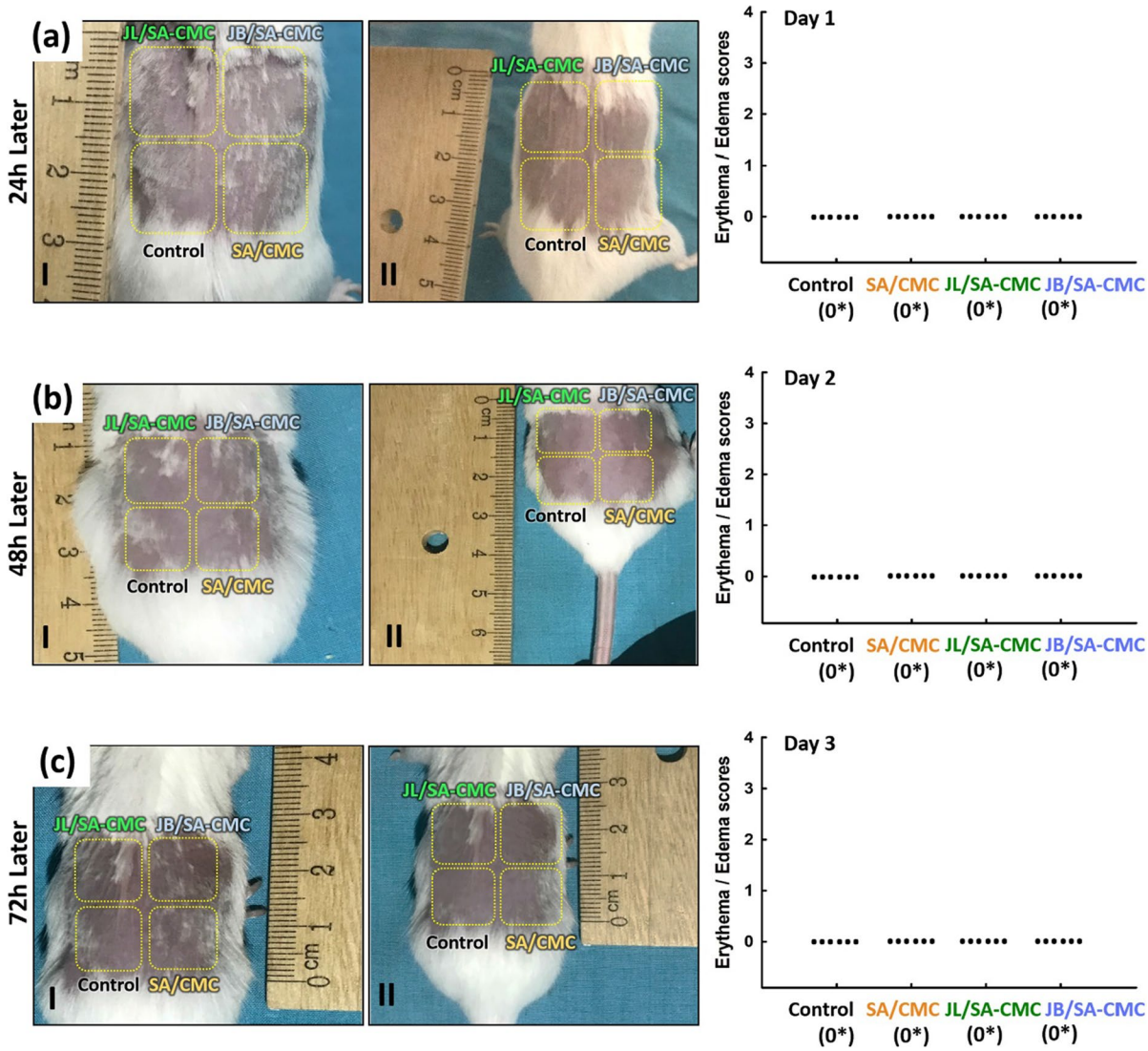

Fig. 5 Primary skin irritation test using hydrogels: erythema and edema scores for each mouse (represented by •, n = 6) at the end of (**a**) days 1, (**b**) days 2 and (**c**) days 3, following twice-daily application of each hydrogel formulation. Hydrogel formulations tested: Control (with PBS solution); SA/CMC hydrogel; JL/SA-CMC hydrogel; JB/SA-CMC hydrogel. All erythema and edema scores were "0" at the end of day 1, 2 and 3. The numbers with superscript letters indicate significant difference at $^{*}P < 0.05$ ($^{*}P < 0.05$ compared with control) as analyzed by Student's T-test

Conclusions

Different hydrogels based on sodium alginate and carboxymethyl cellulose combined have been studied with extracts of juniper leaves and berries have been studied. It has been found that the combination of these components at structural level is dominated by a strong relationship between their functional groups, which increases their compatibility and homogeneity. Moreover, the hydrogels containing berry extracts have shown antibacterial activity against *S. aureus*, while those with leaf extracts have shown an inhibition potential against *P. vulgaris*. Additionally, it has been observed that the combination of extracts reduces the toxicity of the hydrogels, demonstrating their safety with values up to 17.32 $\mu g.mL^{-1}$ as a safe dose when there is direct cell exposure. The adhesion of these hydrogels to a

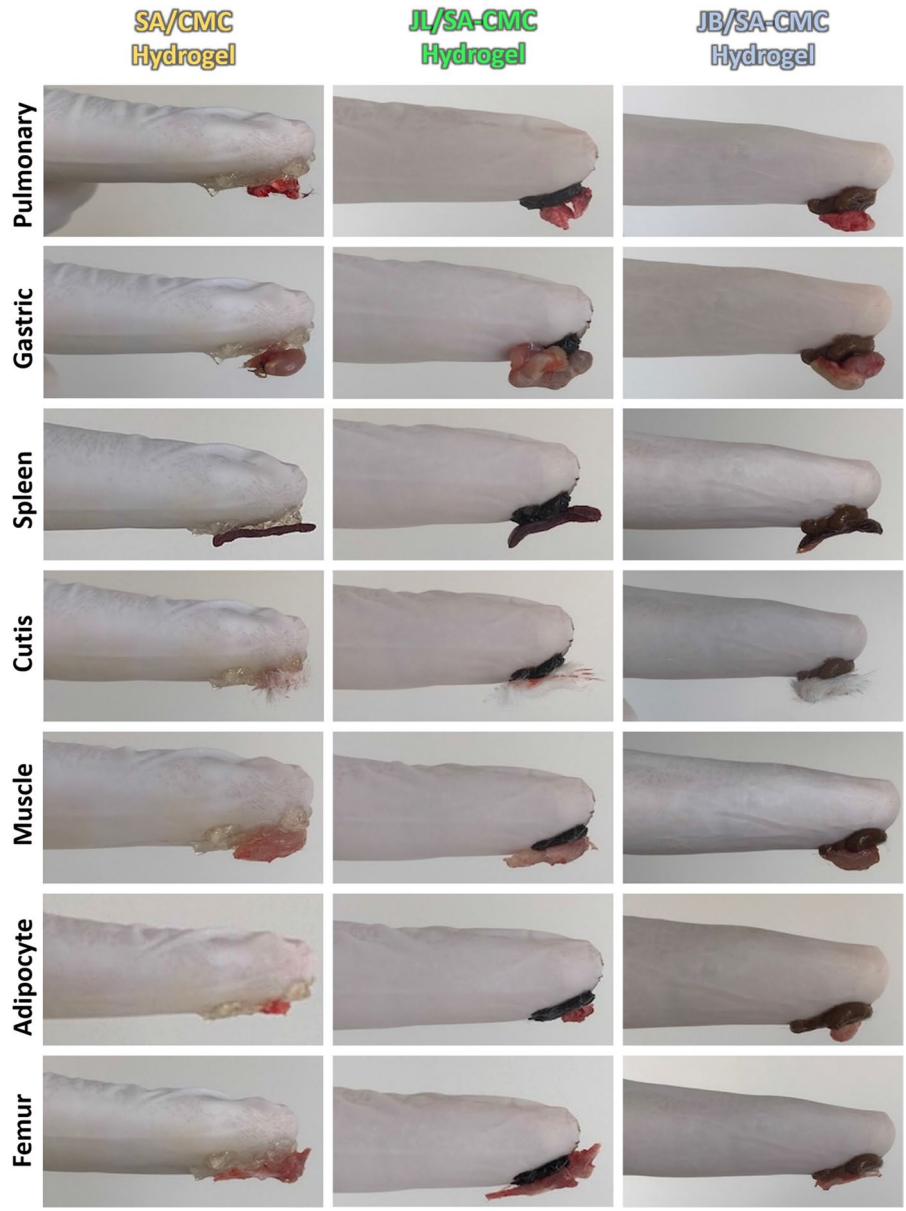

Fig. 6 Adhesion of SA/CMC, JL/SA-CMC, and JB/SA-CMC hydrogels to various biological tissues, including pulmonary, gastric, spleen, cutis, muscle, adipocyte, and femur

variety of tissues has been found to be feasible, as there are no contraindications to their application in any of the evaluated tissues. The invivo model has shown adequate results, demonstrating the safety of these compounds. Therefore, the future and scale-up of these hydrogel combinations are promising for future research in human models, in order to explore and expand their development as an alternative therapeutic treatment or as a scientific basis for future medical applications.

Author contributions KEÖ and CDB-M conducted the biomaterial synthesis and chemical characterization of the materials. İŞ performed the animal experiments with the help of KEÖ and CDB-M. CH and ED analyzed the in vitro and in vivo experiments with the help of İŞ contributed to data interpretation, discussion, and critical comments on shaping the manuscript. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

Ethical approval This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Sivas Cumhuriyet University (26.26.10.2022/65202830-050.04.04-681).

References

Abdellatif Soliman SM, Sanad MF, Shalan AE (2021) Synthesis, characterization and antimicrobial activity applications of grafted copolymer alginate-g-poly (N-vinyl imidazole). RSC Adv 11(19):11541–11548. https://doi.org/10.1039/D1RA01874D

Al-Attar AM, Alrobai AA, Almalki DA (2017) Protective effect of olive and juniper leaves extracts on nephrotoxicity induced by thioacetamide in male mice. Saudi J Biol Sci 24(1):15–22. https://doi.org/10.1016/j.sjbs.2015.08.013

Asadpoor M, Ithakisiou GN, van Putten JPM, Pieters RJ, Folkerts G, Braber S (2021) Antimicrobial Activities of alginate and chitosan oligosaccharides against *Staphylococcus aureus* and group B Streptococcus. Front Microbiol 12:1–15. https://doi.org/10.3389/fmicb.2021.700605

Bajac J, Nikolovski B, Lončarević I, Petrović J, Bajac B, Đurović S, Petrović L (2022) Microencapsulation of juniper berry essential oil (*Juniperus communis* L.) by spray drying: microcapsule characterization and release kinetics of the oil. Food Hydrocoll. https://doi.org/10.1016/j.foodhyd.2021.107430

Bigdarvishi AH, Shirzadeh P, Shahdadian S, Ashrafi F (2021) Antibacterial properties of PEO/PVA nanofibers containing Juniper leaf extract. Res J Biotechnol 16(12):80–86. https://doi.org/10.25303/1612rjbt8086

Cardenas-Jiron G, Leal D, Matsuhiro B, Osorio-Roman IO (2011) Vibrational spectroscopy and density functional theory calculations of poly-D-mannuronate and heteropolymeric fractions from sodium alginate. J Raman Spectrosc 42(4):870–878. https://doi.org/10.1002/jrs.2760

Clinical and Laboratory Standards Institute (CLSI) (2020) Performance standards for antimicrobial susceptibility testing-M100-S24. Clinical and Laboratory Standards Institute, USA, pp 1–230

Deepa B, Abraham E, Pothan LA, Cordeiro N, Faria M, Thomas S (2016) Biodegradable nanocomposite films based on sodium alginate and cellulose nanofibrils. Materials (basel) 9(1):1–11. https://doi.org/10.3390/ma9010050

Ghobril C, Grinstaff MW (2015) The chemistry and engineering of polymeric hydrogel adhesives for wound closure: a tutorial. Chem Soc Rev 44:1820–1835. https://doi.org/10.1039/C4CS00332B

Habibi N (2014) Preparation of biocompatible magnetite-carboxymethyl cellulose nanocomposite: Characterization of nanocomposite by FTIR, XRD, FESEM and TEM. Spectrochim Acta Part A 131:55–58. https://doi.org/10.1016/j.saa.2014.04.039

Han X, Parker TL (2017) Anti-inflammatory activity of Juniper (*Juniperus communis*) berry essential oil in human dermal fibroblasts. Cogent Med 4(1):1306200. https://doi.org/10.1080/2331205x.2017.1306200

Han Y, Wang L (2017) Sodium alginate/carboxymethyl cellulose films containing pyrogallic acid: physical and antibacterial properties. J Sci Food Agric 97(4):1295–1301. https://doi.org/10.1002/jsfa.7863

Horng-Huey K, Yi-Ting T, Ming-Hong Y, Chun-Ching L, Chan-Jung L, Tsung-Han Y, Chiang-Wen L, Feng-Lin Y (2013) Norartocarpetin from a folk medicine Artocarpus communis plays a melanogenesis inhibitor without cytotoxicity in B16F10 cell and skin irritation in mice. BMC Complement Altern Med 13:1–12. https://doi.org/10.1186/1472-6882-13-348

Hou L, Wu P (2018) Exploring the hydrogen-bond structures in sodium alginate through two-dimensional correlation infrared spectroscopy. Carbohydr Polym 205:420–426. https://doi.org/10.1016/j.carbpol.2018.10.091

Huq T, Salmieri S, Khan RA, Tien C, Fraschini RB, C, Bouchard C, Uribe-Calderon J, Kamal MR, Lacroix M, (2012) Nanocrystalline cellulose (NCC) reinforced alginate based biodegradable nanocomposite film. Carbohydr Polym 90(4):1757–1763. https://doi.org/10.1016/j.carbpol.2012.07.065

Hurtado A, Aljabali AAA, Mishra V, Tambuwala MM, Serrano-Aroca Á (2022) Alginate: enhancement strategies for advanced applications. Int J Mol Sci 23(9):4486. https://doi.org/10.3390/ijms23094486

Ivanova DI, Nedialkov PT, Tashev AN, Olech M, Nowak R, Ilieva YE, Kokanova-Nedialkova ZK, Atanasova TN, Angelov G, Najdenski HM (2021) Junipers of various origins as potential sources of the anticancer drug precursor podophyllotoxin. Molecules 26(17):5179. https://doi.org/10.3390/molecules26175179

Ivanova DI, Tashev AN, Nedialkov PT, Ilieva YE, Atanassova TN, Olech M, Nowak R, Angelov G, Tsvetanova FV, Iliev IA, Kokanova-Nedialkova ZK, Yotov GH (2018) Antioxidant and antiproliferative activity of *Juniperus* L. species of Bulgarian and foreign origin and their anticancer metabolite identification. Bulg Chem Commun 50(1):144–150

Jeong D, Joo SW, Hu Y, Shinde VV, Cho E, Jung S (2018) Carboxymethyl cellulose-based superabsorbent hydrogels containing carboxymehtyl β-cyclodextrin for enhanced mechanical strength and effective drug delivery. Eur Polym J 105:17–25. https://doi.org/10.1016/j.eurpolymj.2018.05.023

Jibry N, Murdan S (2004) In vivo investigation, in mice and in man, into the irritation potential of novel amphiphilogels

being studied as transdermal drug carriers. Eur J Pharm Biopharm 58(1):107–119. https://doi.org/10.1016/j.ejpb.2004.02.013

Kacaniova M, Dincheva I, Radoukova T, Semerdjieva IB, Astatkie T, Schlegel V (2018) Essential oil composition, antioxidant and antimicrobial activity of the galbuli of six juniper species. Ind Crops Prod 124:449–458. https://doi.org/10.1016/j.indcrop.2018.08.013

Kamoun EA, Kenawy ERS, Tamer TM, El-Meligy MA, Mohy Eldin MS (2015) Poly (vinyl alcohol)-alginate physically cross-linked hydrogel membranes for wound dressing applications: characterization and bio-evaluation. Arab J Chem 8(1):38–47. https://doi.org/10.1016/j.arabjc.2013.12.003

Kesbiç OS (2019) Effects of juniper berry oil on growth performance and blood parameters in common carp (*Cyprinus carpio*). Aquac Res 50:342–349. https://doi.org/10.1111/are.13908

Klančnik A, Zorko Š, Toplak N et al (2018) Antiadhesion activity of juniper (*Juniperus communis* L.) preparations against Campylobacter jejuni evaluated with PCR-based methods. Phyther Res 32:542–550. https://doi.org/10.1002/ptr.6005

Kumar L, Brice J, Toberer L, Klein-Seetharaman J, Knauss D, Sarkar SK (2019) Antimicrobial biopolymer formation from sodium alginate and algae extract using aminoglycosides. PLoS ONE 14(3):e0214411. https://doi.org/10.1371/journal.pone.0214411

Lan W, He L, Liu Y (2018) Preparation and properties of sodium carboxymethyl cellulose/sodium alginate/chitosan composite film. Coatings 8(8):291. https://doi.org/10.3390/coatings8080291

Lantto TA, Colucci M, Závadová V, Hiltunen R, Raasmaja A (2009) Cytotoxicity of curcumin, resveratrol and plant extracts from basil, juniper, laurel and parsley in SH-SY5Y and CV1-P cells. Food Chem 117(3):405–411. https://doi.org/10.1016/j.foodchem.2009.04.018

Larosa C, Salerno M, Silva de Lima J, Meri RM, Fernandes de Silva M, Bezerra de Calvalho L, Converti A (2018) Characterization of bare and tannase-loaded calcium alginate beads by microscopic, thermogravimetric, FTIR and XRD analyses. Int J Biol Macromol 115:900–906. https://doi.org/10.1016/j.ijbiomac.2018.04.138

Li B, Liu J, Lyu F, Deng Z, Yi B, Du P, Yao X, Zhu G, Xu Z, Lu J, Li YY (2022) Mineral hydrogel from inorganic salts: biocompatible synthesis, all-in-one charge storage, and possible implications in the origin of life. Adv Funct Mater 32:2109302. https://doi.org/10.1002/adfm.202109302

Minero F, Diaz LB (2017) Estudio botánico y farmacéutico de productos con aplicación en cosmética y cuidado de la piel. Ars Pharm 58(4):175–191. https://doi.org/10.4321/s2340-98942017000400005

Och M, Och A, Cieśla Ł, Kubrak T, Pecio Ł, Stochmal A, Kocki J, Bogucka-Kocka A (2015) Study of cytotoxic activity, podophyllotoxin, and deoxypodophyllotoxin content in selected *Juniperus* species cultivated in Poland. Pharm Biol 53(6):831–837. https://doi.org/10.3109/13880209.2014.943246

Pandey S, Tiwari S, Kumar A, Niranjan A, Chand J, Lehri A, Chauhan PS (2018) Antioxidant and anti-aging potential of Juniper berry (*Juniperus communis L.*) essential oil in *Caenorhabditis elegans* model system. Ind Crops Prod 120:113–122. https://doi.org/10.1016/j.indcrop.2018.04.066

Puscaselu RG, Lobiuc A, Dimian M, Covasa M (2020) Alginate: From food industry to biomedical applications and management of metabolic disorders. Polymers (basel) 12(10):2417. https://doi.org/10.3390/polym12102417

Qu J, Zhao X, Liang Y, Zhang T, Ma PX, Guo B (2018) Antibacterial adhesive injectable hydrogels with rapid self-healing, extensibility and compressibility as wound dressing for joints skin wound healing. Biomaterials 183:185–199. https://doi.org/10.1016/j.biomaterials.2018.08.044

Raasmaja A, Stenius U, Ghalali A (2019) The water extract of *Juniperus communis l.* Induces cell death and sensitizes cancer cells to cytostatic drugs through p53 and PI3K/AKt pathways. Int J Mol Sci 20(9):1–9. https://doi.org/10.3390/ijms20092054

Radoukova T, Zheljazkov VD, Semerdjieva I, Dincheva I, Stoyanova A, Kačániová M, Marković T, Radanović D, Astatkie T, Salamon I (2018) Differences in essential oil yield, composition, and bioactivity of three juniper species from Eastern Europe. Ind Crops Prod 124:643–652. https://doi.org/10.1016/j.indcrop.2018.08.012

Semerdjieva I, Zheljazkov VD, Radoukova T et al (2021) Biological activity of essential oils of four juniper species and their potential as biopesticides. Molecules 26:6358. https://doi.org/10.3390/molecules26216358

Shao W, Liu H, Liu X, Wang S, Wu J, Zhang R, Min H, Huang M (2015) Development of silver sulfadiazine loaded bacterial cellulose/sodium alginate composite films with enhanced antibacterial property. Carbohydr Polym 132:351–358. https://doi.org/10.1016/j.carbpol.2015.06.057

Sun Z, Li Z, Qu K, Zhang Z, Niu Y, Xu W, Ren C (2021) A review on recent advances in gel adhesion and their potential applications. J Mol Liq 325:5254. https://doi.org/10.1016/j.molliq.2020.115254

TAPPI (1988) TAPPI Press, Atlanta, USA. https://tappi.micronexx.com/CD/TESTMETHODS/T264.pdf. Accessed 19 Dec 2021

Tyliszczak B, Drabczyk A, Kudłacik-Kramarczyk S et al (2019) In vitro biosafety of pro-ecological chitosan-based hydrogels modified with natural substances. J Biomed Mater Res 107A:2501–2511. https://doi.org/10.1002/jbm.a.36756

Xiaoxuan T, Xinyi G, Yaling W, Xiaoli C, Jue L, Yumin Y (2020) Stable antibacterial polysaccharide-based hydrogels as tissue adhesives for wound healing. RSC Adv 10(29):17280–17287. https://doi.org/10.1039/D0RA02017F

Yaman T, Uyar A, Kömüroğlu AU, Keleş ÖF, Yener Z (2021) Chemopreventive efficacy of juniper berry oil (*Juniperus communis* L.) on azoxymethane-induced colon carcinogenesis in rat. Nutr Cancer 73(1):133–146. https://doi.org/10.1080/01635581.2019.1673450

Zeng Q, Han Y, Li H, Chang J (2014) Bioglass/alginate composite hydrogel beads as cell carriers for bone regeneration. J Biomed Mater Res Part B 102B:42–51. https://doi.org/10.1002/jbm.b.32978

Zheljazkov VD, Semerdjieva IB, Dincheva I, Kacaniova M, Astatkie T, Radoukova T, Schlegel V (2017) Antimicrobial and antioxidant activity of *Juniper galbuli* essential oil constituents eluted at different times. Ind Crops Prod 109:529–537. https://doi.org/10.1016/j.indcrop.2017.08.057