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RESEARCH PAPER

Preparation and characterization of gelatin/ sodium alginate with thymus Kotschyanus hydrogel for wound healing, In Vitro study

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ABSTRACT

Objective(s): Gelatin / Sodium Alginate Thymus kotschyanus (GE/SA T. *kotschyanus*) hydrogel was prepared for use as a wound healing.

Materials and Methods: The hydrogel was prepared by casting and cross-linking method and the physicochemical and the antimicrobial properties of the hydrogels were determined.

Results: The GE/SA hydrogel showed high mechanical properties, while the addition of the extract to the hydrogel led to the relaxation of the polymer network and thus increased the elasticity of the dressing, and it had a good biodegradation. The compatibility of hydrogels with fibroblast cells was confirmed by MTT assay. Also, GE/SA T. *kotschyanus* hydrogels were found to be excellent Hemocompatibile. Moreover, these hydrogels exhibited the antibacterial activity against Pseudomonas aeruginosa, and Escherichia coli. In addition, these hydrogels showed the best antifungal effect against *Candida albicans*.

Conclusion: The GE/SA T. *kotschyanus* hydrogels had the potential for use in wound dressing.

Keywords: *Hydrogel, Gelatin, Sodium Alginate, Thymus kotschyanus, Wound healing*

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INTRODUCTION

Skin is a primary barrier against external pathogens. Therefore, it plays a main role in health. Skin infections are conditions caused by the invasion of harmful microorganisms into the skin [1]. They can range from mild to severe and often require effective treatment. In addition to eliminating bacteria with antibiotics, the treatment should also have wound healing properties [2].

Overall, wound healing typically involves a series of processes, including inflammation, propagation, remodeling, and scar formation [3]. Traditional wound dressing serves as a temporary barrier to stop bleeding and is not effective in preventing infection. Any novel biomaterials identified could help improve the wound healing process [4]. An ideal wound healing should be able to absorb excess secretions, and protect from infection [5].

Hydrogels have obtained the most attention in wound dressing because of various benefits, good biocompatibility, not adherence with wound tissue. In addition, These hydrogels are able to cool the wound site, leading to reduced pain for patients [6]. Natural polymer-based hydrogels, instead of alginate, hyaluronic acid and gelatin, have excellent swelling ability and non-toxic, biocompatible properties [7].

The sequence arginine-glycine-aspartic acid (RGD) is known as gelatin (GE), which enhances

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cell adhesion, proliferation, and differentiation. Gelatin-based hydrogels are transparent, and have the capacity to absorb excess secretions, which shows interest in wound dressing applications [8].

Sodium alginate (SA) is a non-toxic natural polysaccharide from brown algae that is widely used in the pharmaceutical industry [9]. Among the different strategies to induce gelation in alginate hydrogels, ionic binding is the most common. One of the most used agents for crosslinking alginate is $CaCl_2$. Sodium alginate has a high carboxyl group content and also high affinity for divalent cations such as Ca_2^+ ions [10].

Thymus kotschyanus is a family *Lamiaceae* [11]. *T. kotschyanus* is one of *Thymus* species. *T. kotschyanus* are used as an aromatic ingredient, spice or herbal tea is well-known [12]. Medicinal plants have a number of biological compounds, like, saponins, essential oils, alkaloids, flavonoids, coumarins, phenols and tannins, which provide them with pesticide , antibacterial, and antifungal [13, 14].

Recently, *T. kotschyanus* has been considered due to its potential applications, such as pharmaceutical and cosmetic industries [15, 16]. The plant has been reported useful in cases of different infections [17].

In the present study, the GE/SA *T. kotschyanus* hydrogel was evaluated for wound dressing.

MATERIALS AND METHODS

Materials

Gelatin and sodium alginate (Mv = 1.2 × 10^5, μ = 280 mPa s) were procured from Shanghai Chemical Reagent Co. (China). Calcium chloride (CAS No. 10043-52-4) was obtained from Sigma-Aldrich Co. (USA). Phosphate-buffered saline (PBS) and ethanol (C2H5OH, 98% purity) were provided by Merck (Germany). Fresh samples of the plant *T. kotschyanus* were purchased from a local market and identified by the Department of Botany at Razi University (HRU) in Kermanshah, Iran, under identification number 1561. Mouse fibroblast cells (L-929), as well as strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*, were supplied by the Pasteur Institute of Iran.

Plant extract

T. kotschyanus was minced into small fragments and then ground into a fine powder. 25 g of this powder was extracted using 500 ml of 70% ethanol at a temperature of 40°C for a duration of 72 hours in a Soxhlet extractor. The extract was then concentrated through the use of a rotary evaporator. Finally, it was dried for a period of 3 to 4 days before being utilized in subsequent experiments [18].

Preparation of Gelatin /Alginate solution

The initial step involved preparing a 2% weight-volume solution of gelatin and sodium alginate separately. Afterwards, gelatin/ sodium alginate 80/20 was mixed and placed on a stirrer for 24 h. Finally, a uniform solution of two polymers was prepared.

Synthesis of hydrogels containing T. kotschyanus extract

Several concentrations of solution *T. kotschyanus* extract were added and mixed for 60 min at room temperature with a stirrer until complete dissolution was achieved. Eventually, the entire hydrogel solution was transferred to a Petri dish and allowed to dry. Following the drying process, the material was immersed in a 1% calcium chloride ($CaCl_2$) solution for a period of 15 min, acting as a crosslinker for the sodium alginate. In Fig. 1 Preparation and Synthesis GE/

Fig.1. Schematic synthesis of GE/SA / T. kotschyanus hydrogel

SA/*T. kotschyanus* hydrogel was shown.

Hydrogel characterization

Swelling studies

This test was performed to estimate the adsorption capability of GE/SA hydrogel containing *T. kotschyanus* extract (the prepared hydrogel). Initially, the weight of the dry hydrogel and the hydrogel (which was placed in PBS solution at 37°C for 5 min) was measured [19]. The inflation rate of the sample was determined in the time intervals) 30, 60, 90, 120, 150, 180 and 210 min). Finally, the sample was weighed and the swelling rate of samples was computed by following equation No. 1. (W_0: dry weight and W_1: weight of the samples after absorbing water)

(1)

$$Swelling\ ratio\ (\%) = (\frac{W_1 - W_0}{W_0}) \times 100$$

Biodegradability studies

The degradation test hydrogel (1 × 1cm) in 10 mL of PBS solution (pH 7.4) was done. The initial weight of hydrogel was measured and placed in Falcon (10 ml of PBS solution) and an incubator at 37°C. The hydrogel was removed from PBS in 1, 3, 5, 7, 14, days and placed in the oven for 2 h, and weighed again [20]. The weight loss of hydrogel was measured before and after degradation under sterile conditions by equation No. 2. (Wi: initial weight and W_f: final weight).

(2)

$$\text{Degradation ratio } (\%) = \left(\frac{W_i - W_f}{W_i}\right) \times 100$$

Water vapor transmission rate

A diameter of 20 mm from the Hydrogels GE/SA Hydrogel (control), GE/SA /*T. kotschyanus* was fixed on the beaker containing 25 mL of deionized water, separately. Additionally, The same tube without a cap was considered as a control. The first test tube was weighed with water and a proven sample. The hydrogel sample was then incubated for 24 h at 37°C and 40% humidity [21]. After 24 h, it was weighed again, and WVTR was calculated by following equation No. 3.

W_i (initial weight (, W_f (final weight), A (area of the mouth of the tube)

(3)

$$\text{WVTR} = \frac{W_i - W_f}{A}$$

Porosity studies

In summary, the hydrogel was submerged in ethanol. The initial volume of ethanol (V_1) and the combined volume of hydrogel and ethanol were measured as V_2. After one hour, the samples were extracted, and the volume of ethanol that remained was recorded as V_3[22].The porosity was calculated using equation No. 4.

(4)

$$\text{P } (\%) = \left(\frac{V_1 - V_3}{V_2 - V_3}\right) \times 100$$

Strength studies

The tensile-strain of the hydrogel was 50 mm/min and the distance between the two jaws of the device was about 3 cm. According to ASTM D00882 standard dimensions of 1×6 cm were evaluated. The percentage of elongation and elastic modulus of the hydrogel was calculated through the stress-strain diagram and the tensile strength [23]. The Strength was calculated using equation No. 5.

(5)

$$TS=Fmax/A$$

Scanning electron microscopy

The morphology of the prepared hydrogel surface was evaluated by scanning electron microscopy (SEM) (TESCAN-Vega3 co Czech Republic). The hydrogels were frozen in liquid nitrogen and broken using a pair of tweezers. The fractured samples were covered with a thin layer of gold on their cross section.

Fourier transform infrared spectroscopy

The functional groups and bonds formed in the hydrogels were measured by Fourier transform infrared (FT-IR) spectroscopy. The hydrogels are ground into thin tablets. FT-IR absorption peaks were detected in the wavelength range -400 to 4000 cm^{-1} and the type of bonds was defined by examining the absorption band.

Hemocompatibility studies

Fresh anticoagulated blood (2.5 mL) was mixed with normal saline (5 mL) and subsequently centrifuged at 9000 rpm for 5 minutes to separate the red blood cells (RBCs). The RBCs were washed three times with normal saline, and then diluted with normal saline (20 mL). A suspension of RBCs (1 mL) was treated with varying concentrations of hydrogel at 100, 200, 300, 400, 500, and 600 µg/mL. The samples were incubated at 37°C for 3 hours, followed by centrifugation at 9000 rpm for 5

minutes. A volume of 100 µL from the supernatant of each sample was transferred to a 96-well plate, and the absorbance of each well was measured using a microplate reader at a wavelength of 577 nm [18]. The hemolytic degree was measured by equation No. 6.

ODs (absorbance), ODnc (absorbance negative control) and ODpc (absorbance positive control).

(6)

$$\text{Hemolysis}\ (\%) = \left(\frac{\text{ODs} - \text{ODnc}}{\text{ODpc} - \text{ODnc}}\right) \times 100$$

Cell culture

Fibroblast cell lines were cultured in DMEM/F12 medium enriched with 10% FBS, 0.25 µg/mL amphotericin, 100 µg/mL streptomycin, and 100 U/mL penicillin. The culture medium was replaced every two days to maintain over 80% confluence. Subsequently, the cells were trypsinized and plated at a density of 2 × 10^3 cells per well in 96-well plates.

Cytotoxicity assay

The cytotoxic effects of the blank hydrogel (GE/SA) and the hydrogel infused with *T. kotschyanus* (GE/SA/*T. kotschyanus*) were evaluated through an MTT assay. The cells were categorized into four groups: G1: control (fibroblast cell line), G2: Blank Hydrogel (GE/SA), and G3: Hydrogel with *T. kotschyanus* (GE/SA/*T. kotschyanus*). The media from both the control and experimental groups were rinsed with sterile PBS to eliminate any components from the test composites. Subsequently, 50 µl of MTT solution (5 mg/ml PBS) was introduced to each well and incubated for 4 hours at 37°C in a humidified environment containing 5% CO2. After the incubation, 200 µl of DMSO was added to each well to dissolve the colored formazan produced. The absorbance at 570 nm was measured using an absorbance plate reader (Lonza BioTek ELx808 Absorbance Plate Read), The viability percentage was measured by equation No. 7.

(7)

$$\text{Cell viability}\ (\%) = \frac{A570\ (sample)}{A570(control)} \times 100$$

Antibacterial and antifungal *test*

In this study, *E. coli* ATTC 25922, *P. aeruginosa* ATTC 27853 (Gram negative) and *S. aureus* ATTC 25923 (Gram positive) was used for antibacterial test, Also, *C. albicans* ATTC 10231 was employed for antifungal test. These tests were evaluated by disk diffusion method by using Mueller–Hinton Agar (MHA) medium. Afterwards, 100 µl of prepared bacterial or fungal suspension (10^8 CFU/mL) was spread on an agar plate and then the hydrogels were cut into circles of 1 cm diameter, placed on the agar plate, and then incubated at 37°C for 24 h. The inhibition zone was measured in millimeters (mm). Gentamicin (20 µg/disk) disks were used as a positive control for the antibacterial test.

Statistical analysis

GraphPad Prismv.8.0 software (San Diego, CA). was used for cytotoxicity analysis. Comparisons among groups using a one-way ANOVA. P-values of 0.05 were statistically significant.

RESULTS AND DISCUSSION

Swelling study

The dried hydrogels absorb the solvent and swell immediately after immersion in PBS due to the external pressure exerted by the solvent. As shown in Fig. 2a, the swelling of GE/SA and GE/SA /*T. kotschyanus* hydrogels occurred rapidly

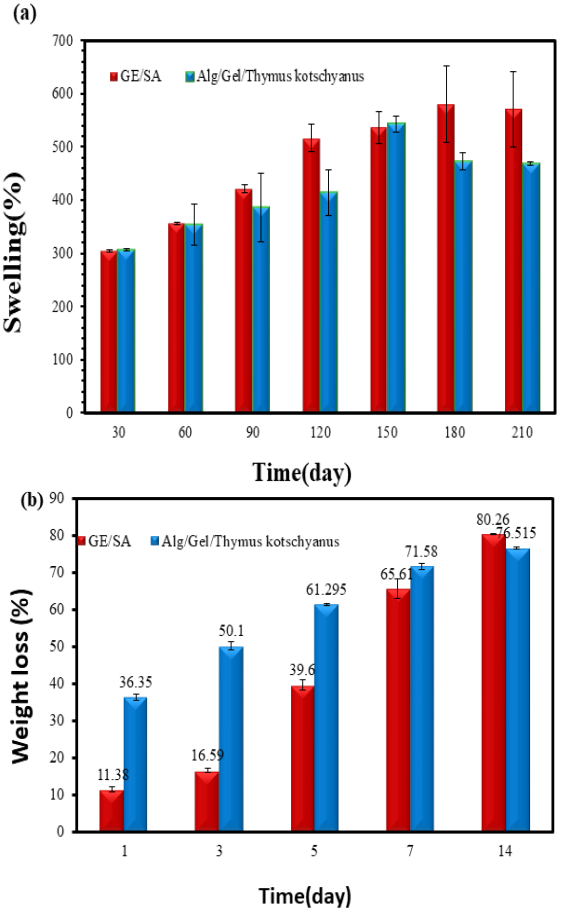

Fig.2. Swelling capacity (a) and degradation (b) of GE/SA and GE/SA / T. kotschyanus hydrogels

in the first 30 min of immersion. Absorption of PBS slowed down after 2 h of immersion but, the swelling of the hydrogels does not change significantly from 3 h to the end of the measurement. This condition represents an equilibrium swelling pressure between two opposing tendencies: a typical increase in entropy due to polymer-solvent mixing and a decrease in entropy due to network expansion [24]. The result obtained from the swelling graph showed that the prepared hydrogels have a good ability to swell. In addition, the hydrogels containing *T. kotschyanus* extract were shown to swell less than the hydrogel without the extract. This is due to the fact that can fill the spaces between polymer chains with organic and inorganic compounds in the extract, and decrease the absorption capacity of modified hydrogels.

Biodegradability of hydrogels

As shown in Fig. 2b, the degradation rate of the samples gradually increased with time. Both alginate and gelatin are physically cross-linked hydrogels that can slowly dissolve during incubation. Therefore, depending on the target tissue, unique cross-linking strategies are needed to combine different polymers to ensure that the hydrogels can be suitable for a specific regenerative application[10]. Amide bonds are fundamentally important in the breakdown of alginate and gelatin scaffolds [25]. The process of natural wound healing is closely related to the degradation of gelatin. The main reason why gelatin degrades rapidly by hydrolysis in aqueous environments is the presence of carboxyl and amine groups [26]. In buffer solution, the degradation is significantly increased with a higher gelatin to alginate ratio, which can be attributed to the sensitivity of gelatin to water caused by the hydrolysis mechanism, as observed in previous similar studies [10, 27]. The increased degradation percentage of extract-containing hydrogel compared to GE/SA in the early days of degradation can be considered due to the presence of extract, which loosens the polymer network, as shown in the mechanical test.

Water Vapor Transmission Rate

Dressings that hold more moisture usually provide suitable environments for faster wound healing compared to dressings that have less moisture holding capacity [28]. Fig. 3a shows the average Water Vapor Transmission Rate (WVTR*)* of samples in 24 h, for GE/SA, GE/SA /*T. kotschy*anus samples. A comparison of hydrogels with the control sample (uncoated bottle) showed that hydrogels can prevent the rapid evaporation of water. However, the water vapor test of the dressing containing the extract showed little change in the reduction of WVTR. The longer diffusion path is the reason for the reduced rate

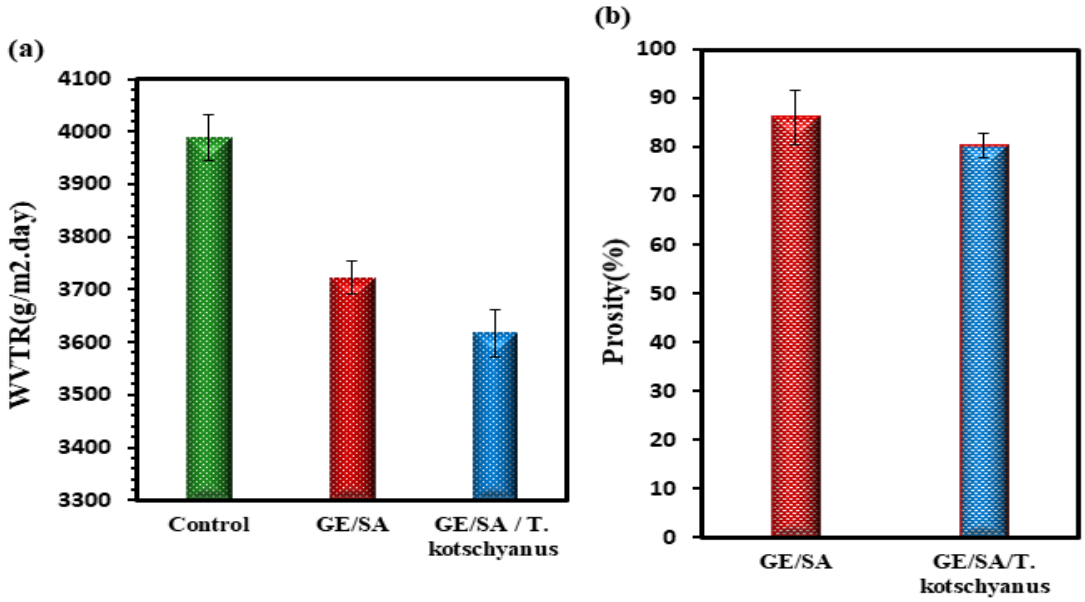

Fig. 3. WVTR (a), Porosity (%) (b) of GE/SA and GE/SA / T. kotschyanus hydrogels

Table 1. Tensile tests

Hydrogel	Tensile strength (MPa)	Young's modulus (MPa)	Elongation at break (%)
GE/SA	0.676±0.0045	1.4977±0.0525	46.1335±1.241375
GE/SA /*T. kotschyanus*	0.177±0.02225	0.4053±0.04285	43.8355±0.995625

of diffusion of water vapor, where the molecules have to move through the hydrogel containing the extract, which, like a filler, reduces the porosity [29]. As shown in the SEM and swelling test for GE/SA hydrogel, the notable increase in WVTR could be attributed to the enhanced porosity of the hydrogel's structure.

Porosity study

The porosity of the hydrogels was investigated using an alcohol displacement method [30]. The hydrogel porosity in the spectrum of 80-86% is shown (Fig. 3b). This rate of porosity can enhance the distribution of nutrients for the cells connected to it.

The mechanical properties test

In order to keep the dressing to remain stable on the wound, sufficient mechanical strength and stiffness without tearing apart is required [31]. The effect of adding *T. kotschyanus* extract on the mechanical properties of GE/SA hydrogel was measured by tensile tests (Table 1). The results showed that tensile strength (σ) and elongation at break (ε) and Young's modulus (E) for GE/SA hydrogel decrease with the addition of extract. In general, stronger chemical bonds in the hydrogel network lead to higher mechanical strength and lower permeability [31]. The presence of plant extract contributes to the relaxation of the polymer network, thereby enhancing the elasticity of the material, which exhibits advantageous mechanical properties. The favorable mechanical characteristics, along with the appropriate elasticity of hydrogels, indicate their potential application in wound dressing [32]. However, hydrogel dressings containing Young's modulus extract have been shown to be within the normal skin range.

Morphology

The cross-sectional images of hydrogels with different magnifications are presented in Fig. 4. It can distinctly be seen that the GE/SA hydrogel exhibits a highly porous 3D network structure with interconnected pores across the cross-section. The surface morphology and porosity of polymer-based wound dressings affect the exudate absorption capacity [10], as observed in the swelling part, more compact pores and smaller pore size increased water absorption. After loading the extract into the hydrogel, differences in pore shape and size were observed, although the hydrogel still maintained its three-dimensional

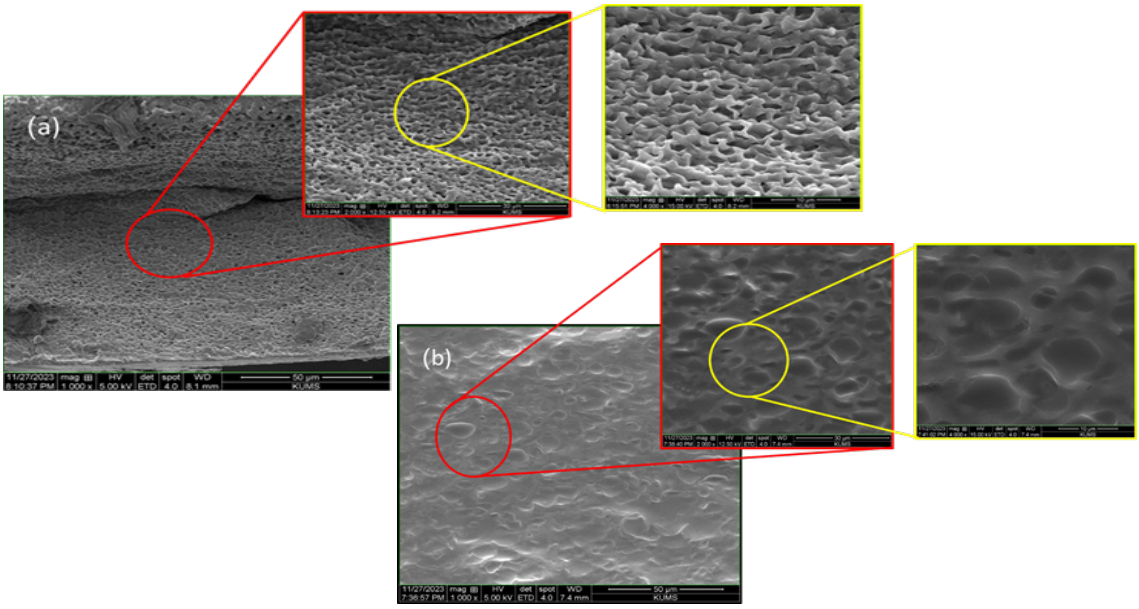

Fig. 4. SEM (a) GE/SA, (b) GE/SA / T. kotschyanus hydrogels in three magnifications of 50, 30 and 10 µm

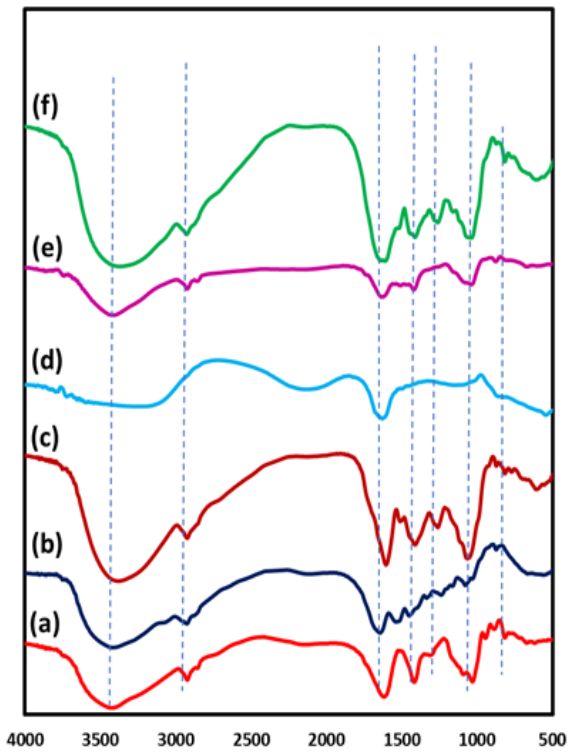

Fig.5. FTIR, a) SA, b) GE, c) T. kotschyanus, d) CaCl2, e) GE/SA, f) GE/SA/ T. kotschyanus

structure, but the hydrogels presented a looser structure (as mentioned in the mechanical section). The cross-sectional images of the hydrogels generally exhibited a structure that was free of cracks, confirming the good compatibility between the GE and SA polymer matrices [30].

FT-IR Analysis

The FT-IR spectrum of the hydrogels was recorded within the range of 500-4000 cm^{-1}. Fig 5 illustrates the FTIR spectrum for alginate, gelatin, *T. kotschyanus*, $CaCl_2$, GE/SA, and GE/SA/*T. kotschyanus*.

The FTIR spectrum of alginate displayed characteristic absorption bands at 3410 cm^{-1} (OH stretching), 1604 cm^{-1} (C=O absorption band of the carboxylic group), 1291 cm^{-1} (C-CH), and 1051-1089 cm^{-1} (C-O stretching), which are indicative of the polysaccharide structure. Additional bands were observed at 1028 cm^{-1} (C-C), 941 cm^{-1} (C-O), 880 cm^{-1} (C-H), and 810 cm^{-1} (Na-O) [33]. Pure gelatin powder exhibited peaks at 3435 cm^{-1}, corresponding to NH and OH stretching. It also revealed the presence of amide I and II bands for the primary amide at 1638 cm^{-1} and 1514 cm^{-1}, respectively. The amide I band is attributed to C=O stretching, while the amide II band corresponds to NH bending vibrations. Furthermore, C-O-C stretching was noted at 1155 cm-1 [34]. The spectrum of *T. kotschyanus* extract presented a prominent peak at 3400 cm^{-1}, associated with the stretching of the N-H bond from amino and hydroxyl groups (O-H stretching), a peak at 2924 cm^{-1} for C-H stretching, and a peak at 1604 cm^{-1} indicating C=O stretching. Additionally, peaks at 1404 and 1255 cm^{-1} were linked to C-C stretching, while the peak at 1054 cm^{-1} was attributed to C-N stretching [35]. A comparison between pure polymers and a mixture of sodium alginate and gelatin (20:80) crosslinked with calcium chloride revealed changes that suggest the formation of strong intermolecular interactions, such as hydrogen bonds and electrostatic attractions between the polymers [36]. Bands at 2920, 2846, 1508, and 1610 cm^{-1} are typically observed in hydrogels. Notably, the spectrum of the GE/SA/*T. kotschyanus* hydrogel displayed a signal intensity that surpassed that of the hydrogel lacking the extract. This heightened intensity suggests the presence of functional groups akin to those identified in the ethanolic extract of *T. kotschyanus*. Furthermore, the absorption patterns recorded in the hydrogel with *T. kotschyanus* extract were markedly similar to those in the hydrogel without the extract. This observation confirms the lack of significant interactions between the matrix that constitutes the hydrogel and the encapsulated material. Essentially, hydrogels incorporating the extract establish an inert environment within the matrix [37].

Hemocompatibility study

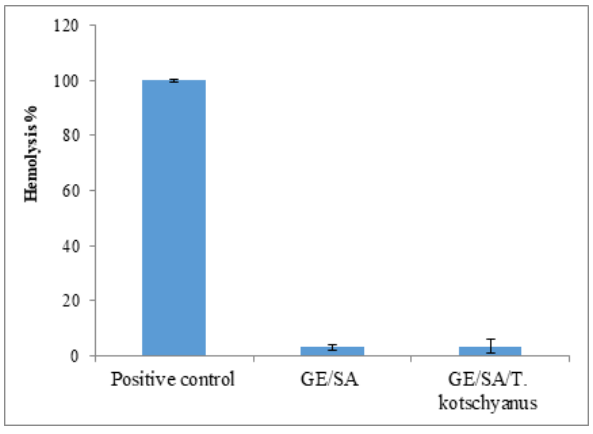

Fig. 6. Results of hemocompatibility study for GE/SA and GE/SA / T. kotschyanus hydrogels Cytotoxicity test

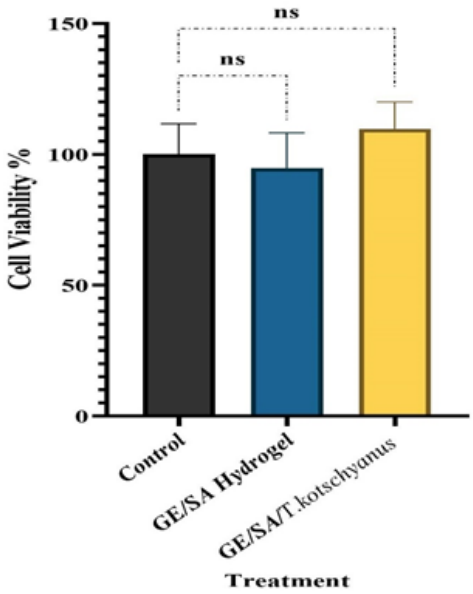

Fig. 7. Cell viability of hydrogels

Wound healing hydrogels must exhibit hemocompatibility, making hemocompatibility a crucial test for their clinical applications [38]. As shown in Fig. 6, the hydrogels demonstrated hemocompatibility when compared to the positive control (Triton X100). The hemolytic rate of the hydrogel was recorded at less than 5%, categorizing it as nontoxic. Furthermore, the bioactive hydrogel (GE/SA/*T.kotschyanus*) showed no signs of hemolysis. Consequently, all hydrogel samples were determined to be hemocompatible.

***In vitro* cytocompatibility**

In a 24h MTT assay, the blank hydrogel (GE/SA), and the hydrogel containing *T. kotschyanus* (GE/SA /*T. kotschyanus*) at the highest concentrations (500 µg/mL) were tested for *in vitro* cytocompatibility on fibroblast cell lines (Fig.7). Our result confirmed that all three groups revealed a slight changing in fibroblast cell lines viability in comparison with the control group (94.72%, and 128.72% vs 100%), so they were fconsidered nontoxic. So, the Hydrogel containing *T. kotschyanus* (GE/SA /*T. kotschyanus*) was cytocompatibile.

Antibacterial and antifungal test

Antibacterial efficacy against pathogens responsible for wound infections, such as *Pseudomonas aeruginosa*, *Escherichia coli* (Gram-negative), and *Staphylococcus aureus* (Gram-positive), was evaluated, with the results quantified by measuring the zones of inhibition in millimeters, (Fig. 8). GE/SA / *T. kotschyanus* hydrogels (2.5%) were extra active against *E. Coli* and *P. argenosa* and less active against *S. aureus* (Table 2). The reason for this phenomenon may be referred to the use of total extract of *T. kotschyanus* and thymol and other antibacterial and biological compound concentrations during hydrogel synthesis that is not suitable for penetrating *S. aureus* cell wall. The polymeric

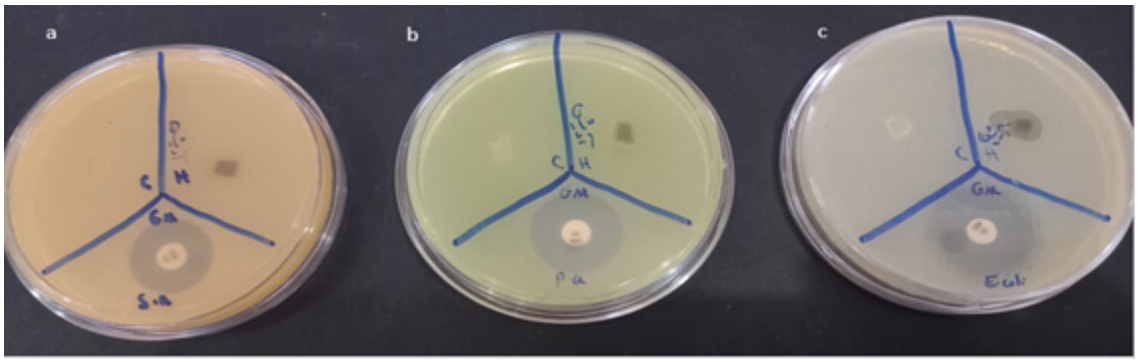

Fig. 8. Antibacterial activity GE/SA / T. kotschyanus hydrogel: a) S. aureus b) P. aeruginosa c) E. coli. Gentamicin (20 µg/disk) disks as positive control (GM), GE/SA hydrogel as negative controls(c).

Table 2. Inhibition zones detected for hydrogel.

Organisms	GM (20 µg/disk) (mm)	GE/SA hydrogel Control (mm)	GE/SA / *T. kotschyanus* hydrogel (mm)
S. aureus	18	0	0
P. aeruginosa	20	0	8
E. coli	25	0	10

component of the hydrogels engages with the cytoplasmic membrane due to strong electrostatic interactions between the negatively charged bacteria and the charged hydrogels containing multifunctional groups. This interaction within the polymeric matrix contributes to the antibacterial properties of the hydrogels. A robust coordination interaction undermines the structural integrity of the bacterial cell wall through the carboxyl and carbonyl groups present in peptidoglycans. Furthermore, the polymeric component of the hydrogel interacts with bacterial DNA, effectively seizing control of bacterial functions, which inhibits bacterial growth and ultimately leads to bacterial death [39, 40]. The antibacterial effect of the synergistic activity of polymer and *T. kotschyanus* is durable. Contamination of the hydrogel is the main challenge, so it must be aseptically and freshly synthesized every time.

On the other hand, The antifungal test against *C. albicans* ATTC 10231 was determined by disk diffusion method, as shown in Fig. 9. GE/SA/ *T. kotschyanus* has a strong antifungal effect (11mm), and this hydrogel could be very useful for preventing of infection by *C. albicans* as a wound dressing.

CONCLUSION

In this study, we prepared hydrogel wound dressing with the aim of producing a safe and biocompatible formulation that is easy to use and does not require a complex, energy-intensive method. Given the antiseptic, antibiotic and antifungal properties of *T. kotschyanus*, its hydroalcoholic extract has been used in dressings. The physicochemical properties of the hydrogels were determined by various spectroscopic and microscopic techniques. The mechanical properties showed good elasticity that was completely flexible and stable. The hydrogels showed good swelling behavior and high porosity as confirmed by SEM images. Hydrogels were shown to be non-cytotoxic against fibroblasts. The antimicrobial and physicochemical properties of hydrogel such as hemocompatibility showed that GE/SA/*T. kotschyanus* was potentially suitable for wound care and treatment.

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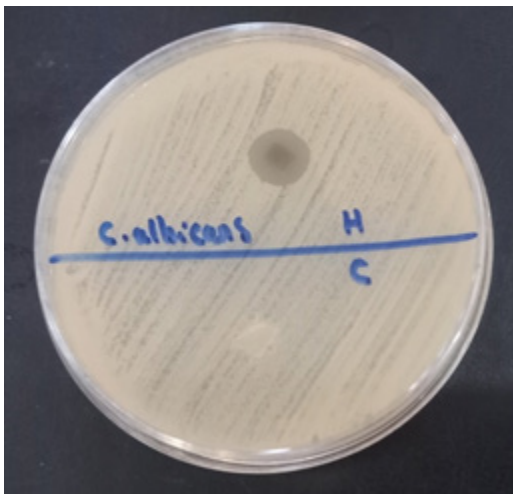

Fig. 9. Antifungal activity, H: GE/SA / T. kotschyanus hydrogel (11mm), C) GE/SA hydrogel (0mm)

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

ETHICAL CONSIDERATION

This study was ethically approved by the Kermanshah University of Medical Sciences, Institutional Review Board (IR.KUMS.AEC.1401.014).

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