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# Preparation of a naturally driven cotton wound dressing via honey, Tragacanth and *Sumac*

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An antibacterial wound dressing with wound healing effects of honey (H), Tragacanth Gum (TG) and *Sumac* (S) (Rhus coriaria L.) has been prepared. The antibacterial properties of five different concentrations of water extracted *Sumac* has been examined to find out the best sample. Ratios of honey and Tragacanth Gum are investigated along with the chosen concentration of *Sumac* in order to find out the optimum compound with desirable antibacterial and healing effects. The results of the well diffusion test indicate antibacterial activities against *S. aureus* and *E. coli* on all samples. Scratch test results demonstrate improvement in the proliferation of fibroblasts on the fabric treated with compounds. The prepared wound dressing accelerates the healing process and eliminates bacterial growth causing wound infection.

**Keywords:** Anti-bacterial properties, Cotton, Honey, *Sumac*, Tragacanth gum, Wound dressing

## 1 Introduction

Wound healing is a complex process, which occurs in the body after an injury, and results in repairing a lost or damaged tissue. Wounds, in general, change the skin structure and the function of affected area. Some microorganisms such as bacteria among others could delay the process of healing [1]. Ideal wound dressing should protect wounds from bacterial infection by providing a moist and restorative setting, which is safe and biocompatible [2, 3]. It should also have a high porosity for vapor penetration, be able to absorb excess body fluids and able to form a sterile barrier against bacteria[4].

A few research articles have reported the use of natural compounds to functionalize cotton fabric for achieving antibacterial activities and according to their test results, the modified fabric has high antibacterial activity against both Gram-positive and Gram-negative bacteria [5-7]. One of the most promising materials with antibacterial effect is *Rhus coriaria*, commonly known as *Sumac*[8]. Numerous studies have reported its antibacterial and antioxidant properties[9-11]. *Rhus coriaria* L. widely grows in Canary Island over the Mediterranean coastline to Iran and Afghanistan [11]. *Sumac* is one of the top 10 herbal antioxidants containing hydrolyzable tannins, gallotannins, volatile oil, flavonoids, anthocyanin, gallic acid, flavones, such as myricetin, quercetin and kaempferol, nitrate and nitrite contents, moisture, oil, protein, fibre, and ash. Additionally, malic, palmitic, stearic, oleic, and linoleic acids are found as the major components of *Sumac* oil [12]. *Rhus cororia* also possesses minerals including K, P, Si, Br, Al, Cu, S, Cl, Pb, Ti, Ca, Mn, Fe, Zn, Sr, Mg, Ba, Cr, Li, N, and V, which are useful in the treatment of different disorders and contribute to various biological processes[13, 14]. In traditional medicine, *Sumac* has been widely used as a natural, safe and cheap antimicrobial resource[10, 13] for treatment of wounds, animal bites, pain, liver disease, sore throat and other diseases[11]. The strong antimicrobial activities of *Sumac* could be attributed to the easy passing of non-dissociated form of weak acids through the cell membrane. This makes *Sumac* an attractive condiment in food industry. The acidic environment caused by *Sumac* in food provides a major survival challenge for various organisms, such as bacteria [15].

The methanol extract of *Sumac* has high inhibitory effect on different strains of both Gram-negative and Gram-positive bacteria[12]. Some studies show that generally, Gram-positive bacteria are more sensitive against antibacterial agents than Gram-negative strains[16, 17]. Thus, *Sumac* water extract showed more resistance on Gram-positive bacteria than on Gram-negative ones[18].

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The second material (honey) has been known to possess wound-healing effect as well as antimicrobial properties[19-21]. Honey has been used for medical purposes since ancient times [22]. Honey is generally a highly soluble sugar solution comprises 38.2 % fructose, 31 % glucose, 17.1 % water, 7.2 % maltose, 2.5-4.4 % carbohydrates, 1.5 % sucrose and 0.5-0.7 % mineral salts, vitamins and enzymes[23]. It has anti-bacterial and antifungal properties, along with a high acidity, which helps to reduce dampness of the wounded area [24, 25]. Honey can eliminate dead tissues with its chemical and enzymatic functions and accelerates the formation of new tissue in the wound. It had been widely used in medical procedures such as burns; pain management, fighting infections; sterilizing wounds, accelerating the healing process and reducing inflammation[1]. It has also a better choice to utilize honey for the treatment of wounds in comparison with silver (as an antibacterial agent [26]), because of the fact that silver delays re-epithelialization which is an important phase of wound healing[27]. Furthermore, using electrospun Manuka honey/silk fibroin fibrous matrices was proven to have positive effects on wound healing [1]. Manuka honey (MH) has unique properties such as anti-inflammatory [28], anti-microbial activities [29], tissue growth [30, 31] and reducing pain of patients [1]. MH provides exclusive antibacterial activities compared to conventional kinds of honey, partly due to methylglyoxal (MGO) [32, 33]. *In vivo* wound healing assays have proven the biocompatibility of the silk fibroin and antibacterial properties of MH along with their wound healing properties [1, 32]. Some other forms of honey such as Leptospermum honey was successfully used for the treatment of neonatal wounds [34], which is not the focus of the current study.

Biopolymers, such as polysaccharides, chitosan [35, 36], alginate [37] and glycosaminoglycans have hydrogel properties and were also used as an effective wound dressing [38]. Tragacanth (TG) is a complex natural mixture of polysaccharides and alkaline minerals, extracted from the root of the plant species containing bassorin as water-swellable part and Tragacanthin as water-soluble component [39, 40]. Bassorin forms about 60 - 70 % of TG [41], which can absorb water and form a solution. Bassorin consists of d-xylose, L-fucose, d-galacturonic acid, d-galactose and a very small amount of L-rhamnose. Tragacanthin as a highly branched polymer with L-arabinose is the predominant sugar [42]. TG is considered as a suitable material for skin wound dressings owing to non-allergic effects, and lack of toxicity for human body [38, 43].

The effect of some natural materials such as chitosan, green tea, and honey in treating common wounds have already been studied [25, 35]. However, no study has been conducted on determining the efficacy of cotton wound dressing treated with *Sumac* extraction, Tracaganth and honey altogether. Present investigation is focussed on the application of these three natural products on cotton fabric to prepare a facile and skin friendly wound dressing.

## 2 Materials and Methods

Tragacanth was collected from *Astragalus gummifer* plants, growing in Fars province (Iran). Honey and *Sumac* (*Rhus coriaria*) were collected from the mountains of Azerbaijan and Kordestan areas in Iran. All the above compounds were procured from the local market in Tehran, Iran. A 100 % bleached cotton fabric with 140 $gm^{-2}$, 20 Nm yarn count, 22 yarn $cm^{-1}$ warp and 25 yarn $cm^{-1}$ weft was used as the textile material; purchased from Yazdbaf Co., Yazd, Iran.

### 2.1 Preparation of Samples

First, the bleached cotton fabric was rinsed with tap water. Then, 10×10 cm samples were prepared and washed for 30 min at 60 °C using non-ionic detergent (1 g/L) and finally rinsed with tap water. *Sumac* extracts were prepared with distilled water (WES) at a ratio of 1:4 - 1:8 (WES#1 – WES#5) and placed in an ultrasound bath (100 Hz frequency, 50 °C) for 1 h. The antibacterial effects of all extracts were tested against *E. coli* and *S. aureus* [44, 45] (Table 1).

The most effective ratio, with the highest antibacterial activities, was found to be 1:4 (WES#1). TG solution was prepared at 0.05 % (wt/vol) since more ratios of TG could have a negative effect on the antibacterial activity of other components. TG was completely dissolved after 2 days and a uniform solution was made.

Table 1 — Inhibitory zone diameter of water extracted *Sumac* (WES#1-WES#5) against *E. coli* and *S. aureus*

| Code | *Sumac*:distilled water, g/mL | Zone of inhibition, mm | |
|---|---|---|---|
| | | *E. coli* | *S. aureus* |
| WES 1 | 1:4 | 2.1 | 2.1 |
| WES 2 | 1:5 | 2.0 | 2.0 |
| WES 3 | 1:6 | 1.8 | 1.9 |
| WES 4 | 1:7 | 1.7 | 1.8 |
| WES 5 | 1:8 | 1.6 | 1.7 |

At first, 60:40 WES1:TG solution (compound 1) was prepared and tested to evaluate the antibacterial activities of the combination of TGS with water extracted *Sumac* that showed strong antibacterial activities against *S.aureus* and *E.coli*.

Secondly, 60:20:20 of WES1:TGS:H (compound 2) was prepared and tested similarly. Likewise, compound 2 displayed a higher antibacterial effect, with a higher inhibition zone compared to compound 1 (1.5>1.3 for *E.coli* and 1.8>1.7 for *S.aureus*).

Lastly, both prepared compounds 1 and 2 were coated on the described cotton fabric using the pad-dry method. Compounds were applied on the fabric with 100 % wet pick-up and then dried at 80 °C for 1 h.

#### 2.2 Antibacterial Activity through Agar Well Diffusion Method

In order to determine the antibacterial activities of the solutions against Gram-positive (*S. aureus)* and Gram-negative (*E. coli)* bacteria, agar well diffusion method (semi-quantitative method) was used [44].

Microbial suspension of $1\times10^8$ CFU per mL (equivalent to 0.5 McFarland) was cultured with sterile swabs in three directions in Tryptic Soy Agar. A 6 mm diameter well was then made with a glass Pasteur pipet and 60 λ of solutions were inserted in the wells and finally placed in an incubator at 37 °C for 24 h. The diameter of the inhibition zone was measured with a scale. A larger zone of inhibition usually is indicative of a more potent antimicrobial effect.

#### 2.3 Antibacterial Activities Through Microbial Growth in Suspension

The quantitative test method was performed according to AATCC Test Method 100-2004. To obtain a $1\times10^5$ suspension of bacteria, a micro-organism colony was added to the tube containing the Tryptic Soy broth culture medium using a sterilized loop. The McFarland equivalent suspension absorption peak of 0.5 ($1\times10^8$ CFU/mL) at 600 nm is found 0.08-0.1. The samples were then diluted $10^3$ times and a suspension of $1\times10^5$ CFU/mL was prepared. Circular swatches (4.8 ± 0.1 cm in diameter) were cut from the test fabric and sterilized by autoclave at 121 °C and 15 lb. /inch$^2$ for 20 min in 100 mL containers named "Media-Lab Bottle". Then, 1 ml of the prepared $1\times10^5$ CFU/mL microbial suspension in liquid culture media was placed on swatches and incubated for 24 h at 37 °C. Antimicrobial activities against two bacteria *S. aureus* (Gram-positive, ATCC#25923) and *E. coli* (Gram-negative, ATCC#25922) were examined. After 24 h, the amount of 100 mL normal saline was added to the container and shaken vigorously to produce a balanced osmotic pressure for microorganisms. From each container, which had 100 mL physiological serum, 1 mL microbial suspension of different samples (serial dilution of $10^0$, $10^1$ and $10^2$) was separately transferred on a plate. Then, 15 mL melted Tryptic Soy agar culture medium (45 °C) was added to each sample and simultaneously shaken to mix the microbial suspension in the melted agar. Plates, which were left at room temperature (25 °C), became solid. In the end, the plates were inverted and incubated at 37 °C for 24 h. After incubation, plates were removed from the incubator and studied for the number of colonies. The number of bacteria in each plate was counted and the percentage of microorganism reduction was determined according to following equation:

$$R\% = (A-B)/A*100 \quad \ldots(1)$$

where $A$ is the number of primary colonies (control) in time zero (immediately after inoculation); $B$, the number of colonies after 24 h incubation; and $R$, the percentage reduction in the number of bacterial colonies.

According to ASTM E 2149 standards, a bacterial reduction of less than 50 % is considered insignificant and more than 50 % is acceptable.

#### 2.4 Wound Healing Analysis

Scratch test is an easy and economical method for studying the wound healing properties of substances by measuring the migration of either native cells or transfected cells[46]. In this test, an appropriate extracellular matrix (ECM) of fibroblast and fibronectin as the control unit was coated on 60 mm dishes and incubated overnight at 4 °C. After removing the unbound ECM from the coated dishes, 3 mL bovine serum albumin (2 mg mL$^{-1}$) was added to the dish and left at 37 °C for 1 h. The dishes were then washed using phosphate buffer saline (PBS) and refilled with 3–5 mL media. The versene containing Trypsin was added to the cells and mixed with medium containing serum to re-suspend the growing cells. The solution was taken out and the dish was shaken to separate the cells. A monolayer of cells was prepared through culturing in a 60 mm dish. Then a scratch was created on the cell monolayer and the scratch edge was smoothed by washing with 1 ml of the growth medium and replacing with 5 mL of

specific medium for the *in-vitro* scratch test. A first image of the scratch was recorded by an inverted microscope and the dish was placed in a $CO_2$ incubator at 37 °C for 8–18 h. The second image was then taken through a matching dish with the reference point in the first image. The distances between two sides of the scratch were measured at a certain interval (μm) and the cell migration rate at the time of *t* (Mt %) was calculated using the following equation.

$$Mt\% = [(d_0 - d_t)/d_0) \times 100] \quad \ldots (2)$$

where $d_0$ and $d_t$ are the average distances between two sides of scratch in times of zero and *t*, respectively.

## 3 Results and Discussion

### 3.1 Agar Well Diffusion Test Results

The results of agar well diffusion test are explored by comparing the inhibitory zone of compounds after centrifuging and isolating grains of WES#1 to WES#5 against *E. coli* and *S. aureus*. Figures 1(a) and (b) show that WES#1 to WES#5 are resistant to both bacteria, and the higher concentration of WES indicates the greater resistance. Several researchers indicated the antibacterial effects of *Sumac* [8, 10, 12] and a few claimed greater potency for *Sumac* extraction against Gram-positive than Gram-negative bacteria [18]. The results for WES#3,4 and 5 support the claim as their inhibitory zone is partially more for *S.aureus* than for *E.coli*. (Table 1). Figures 1(c) and (d) also show that compounds 1 and 2 are resistant to both bacteria. The diameter of inhibition zone of compounds 1 and 2 is found 1.3 and 1.5 mm for *S. aureus*, and 1.4 and 1.8 for *E. coli* respectively. The difference is presumably related to the presence of honey with strong antibacterial activity against various bacteria including *S. aureus* and *E. coli* [21, 22, 25, 32, 47]. Raw honey contains compounds which may function as anti-oxidants, such as flavonoids and other polyphenols. Honey with the high degree of osmolality, hydrogen peroxide and non-peroxide components exhibits antibacterial and anti-inflammatory effects [23]. Thus, zone of inhibition of compound 2 is greater than that of compound 1, which indicates greater antibacterial activity.

### 3.2 Antibacterial Activity of Treated Fabrics

AATCC Test Method 100-2004 has also been applied to determine the antibacterial effects of the fabric treated with compounds 1 and 2 against *E. coli* and *S. aureus* (Fig. 2). Figure 2 (a) demonstrates petri

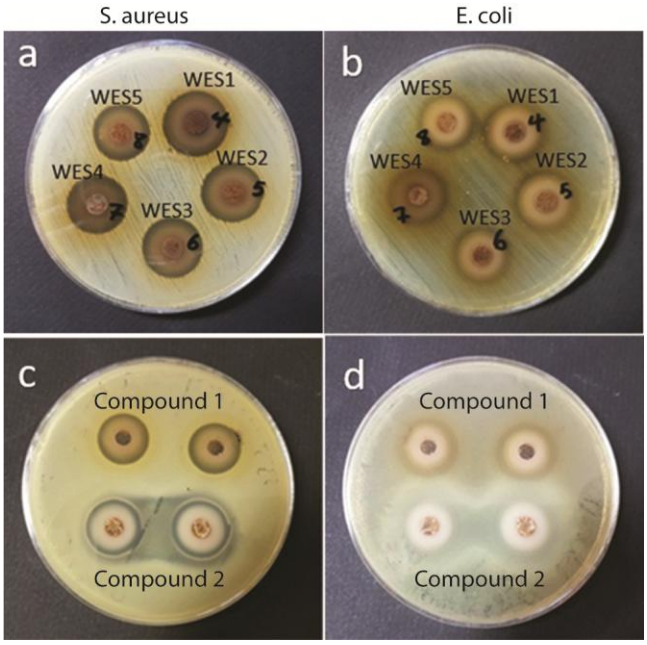


Fig. 1 — Inhibitory zone of WES#1 to WES#5 against *S. aureus* & *E. coli* [(a) & (b)] and inhibitory zone of compounds 1 and 2 [(c) & (d)] against *S. aureus & E. coli*

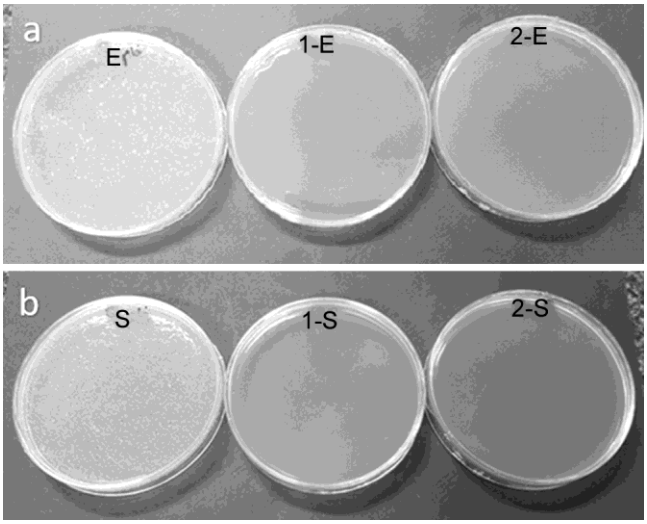


Fig. 2 — Antibacterial efficiency of control and treated fabrics (E) control, (1-E) compound 1, (2-E) compound 2 against *E. coli* (a); and (S) control, (1-S) compound 1, (2-S) compound 2 against *S.aureus* (b)

dishes labeled E, 1-E and 2-E containing control sample, extracted solution from cotton fabric treated with compounds 1 and 2 subsequently against *E. coli*. The reduced number of *E. coli* colonies is 94 % for compound 1 and 96 % for compound 2. However, the efficiency of compound 2 is higher than compound 1 due to honey properties. Figure 2-b shows petri dishes labeled S, 1-S and 2-S containing control sample,

extracted solution from cotton fabric treated with compounds 1 and 2 against *S. aureus*. The reduction of *S. aureus* colonies for compounds 1 and 2 is 95 and 96 %. Therefore, both samples (1-S and 2-S) have antibacterial effects. Similarly, honey enhances the antibacterial activities of compound 2 against *S.aureus*. Since bacterial reduction of all compounds is more than 50 % then according to ASTM E2149, another standard test method for determining the antimicrobial activity compounds 1 and 2 can be considered as acceptable antibacterial agents.

### 3.3 Wound Healing Assay

Scratch test was carried out to examine the effects of prepared compounds on cell migration (fibroblasts). It is an economical and well-developed method to study cell migration and proliferation *in vitro* [46, 48]. According to Figs. 3(c) and (d), compounds 1 and 2 neither enhance the cell growth nor damage them. The high concentrations of solutions appear to be causing difficulty in cellular inhalation system. Solution concentrations should simultaneously maintain their antibacterial properties and sustain respiration of cells. Solutions taken from fabrics impregnated with compounds 1 and 2, unlike solutions of the compounds, indicate significant effects on the cell growth [Figs 3(e) and (f)].

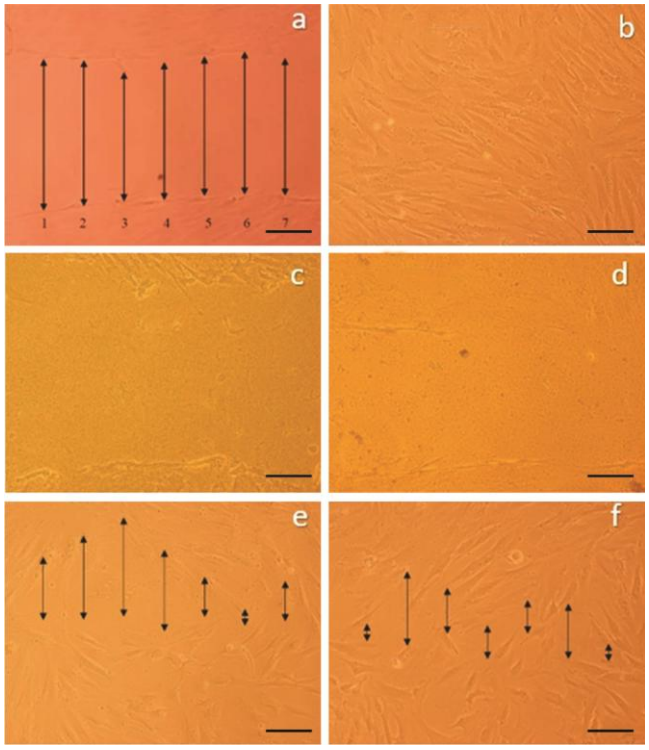


Fig. 3 — Fibroblasts migration by *in vitro* scratch test (a) time 0, (b) after 24 h for control sample, (c) compound 1 after 24 h, (d) compound 2 after 24 h, (e) extracted solution of the fabric treated with compound 1 after 24 h, and (f) extracted solution of the fabric treated with compound 2 after 24 h [scale bar 100 µm]

Table 2 demonstrates the migration percentages of the fibroblasts in 7 different points after 24 h. The average migration rate of fabrics treated with compounds 1 and 2 is 57 and 72 % respectively. The higher migration percentage of compound 2 could be attributed to the wound healing properties of honey [22]. Hydrogen peroxide is the most important agent in honey, and its concentration is determined by relative levels of glucose oxidase, synthesized by the bee and catalase, originating from flower pollen [23]. The activation of glucose oxidase that oxidizes glucose to glucaric acid and $H_2O_2$ contributes to the antimicrobial activity of most types of honey when diluted [21]. Several bacteria such as *E. coli*, *Salmonella*, and *S. pyogenes* have been inhibited by commercial therapeutic honey [49]. Due to high osmolality and sugar content, honey can hinder the growth of microbes [21] and its low moisture content is the reason why yeasts enter their dormant stage and prevent the fermentation process [50]. Based on a study exploring the antimicrobial properties of honey *in vitro*, $H_2O_2$, MGO and an antimicrobial peptide, bee defensin-1 [51], are distinct mechanisms involved in the antibacterial trait of honey [21, 22]. Some researchers reported the ‘‘high-quality evidence’’ and ‘‘unequivocal results’’ of honey as a superior dressing which accelerates healing in treating partial-thickness burns [22].

Another natural material that is widely used for wound healing is TG with remarkable biological properties and low cost, that was chosen to use as a wound healing compound [52]. According to a study regarding wound healing effects of TG on rabbits, the rate of healing is accelerated due to the possible capability of stimulating myofibroblasts contraction, resulting in faster closure of the wound. TG is thought to be effective in proliferation and restoration phases of a wound. Active components of TG (bassorin and tragacanthin) might be the contributing factors for such healing qualities [52].

Numerous research used a combination of natural materials and herbs for wound dressing applications; although, none of them has used *Sumac* and honey with TG. For instance, a film containing silk fibroin, wool keratin, chitosan, and honey was used for a wound dressing application. The antimicrobial efficacy of the film was measured against *S. aureus* and *E. coli*. Different combinations of materials

Table 2 — Migration of cells in 7 points after 24 h for compounds 1 and 2

| Point number | Compound 1 | | | Compound 2 | | |
|---|---|---|---|---|---|---|
| | $d_{0\ comp1}$ mm | $d_{24\ comp1}$ mm | Migration of cells in 7 points after 24 h, % | $d_{0\ comp\ 2}$ mm | $d_{24\ comp\ 2}$ mm | Migration of cells in 7 points after 24 h, % |
| 1 | 1.040 | 0.431 | 58.6 | 1.040 | 0.120 | 88.4 |
| 2 | 1.000 | 0.568 | 43.1 | 1.000 | 0.505 | 49.4 |
| 3 | 0.885 | 0.672 | 24.0 | 0.885 | 0.310 | 64.9 |
| 4 | 0.942 | 0.551 | 41.5 | 0.942 | 0.229 | 75.6 |
| 5 | 0.954 | 0.270 | 71.7 | 0.954 | 0.229 | 75.9 |
| 6 | 0.982 | 0.114 | 88.3 | 0.982 | 0.373 | 62.0 |
| 7 | 0.942 | 0.270 | 71.3 | 0.942 | 0.120 | 87.2 |
| Average | | | 57 | | | 72 |

showed antimicrobial activities against *S. aureus* and *E. coli* based on the agar diffusion test [53]. Also, TG/poly(ε–caprolactone) has been used to make a framework containing different concentrations of PCL for skin wound dressing applications. The antibacterial activity of PCL/TG and TG nanofibres against *S. aureus* and *P. aeroginosa* was examined, indicating resistance against both bacteria. The most organisms could not metabolize the foreign sugars, such as L-arabinose and L-fucose, thus the antibacterial effect might be contributed to the presence of mentioned L-sugars in TG. Also human fibroblast and NIH 3T3 fibroblast cells adhered and proliferated well on PCL/GT supports [52].

### 3.4 Cost and Expenses

According to the national honey board, the average retail price of honey is about 16.8 $ per kilogram in 2019 [54]. Hundred grams of tragacanth gum is about 70 $ in Sigma Aldrich, However, the powder price varies from 1 to 80 $ per kilogram. Sumac powder can be obtained for approximately 1 - 10 $ per kilogram. Based on the amount of consumed materials, the price of the finishing materials is about 50 cents per 1 $m^2$ of the used fabric which is very low for a wound dressing with healing effects.

## 4 Conclusion

The findings of the current study clearly show that the prepared compounds can be potentially used in wound dressing with antibacterial properties, which prevent and control infection of wounded skin. WES1 indicates the highest antibacterial effects against *S. aureus* and *E. coli* among the other tested ratios (WES#1 to WES#5). Compounds 1 and 2 show no positive effects on the growth of skin cells. One may conclude that the high concentrations of two solutions have interfered with the skin cells' respiration. The cotton fabrics treated with compounds 1 and 2 improve the cellular proliferation contributed to the growth and multiplication of cells in the scratch. The images of the wound healing test demonstrate a relatively decent wound healing activities on the extracts of the treated fabrics with compounds 1 and 2, as the fibroblast cells migrate towards the center of the scratch and closed the major part after 24 h. The honey-containing compound shows better effects on the growth and proliferation of cells. Consequently, the coating of compound 2 on the cotton fabric may be potentially a better choice for wound dressing and healing applications.

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