Polymer Bulletin
https://doi.org/10.1007/s00289-019-03095-1

ORIGINAL PAPER

The properties of nanofiber scaffolds of polyurethane-*Cinnamomum zeylanicum* against pathogens of *Pseudomonas aeruginosa* and *Staphylococcus aureus*

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Received: 23 April 2019 / Revised: 29 October 2019 / Accepted: 28 December 2019

Abstract

This study designed to investigate the properties of antibacterial nanofiber scaffolds of polyurethane-*Cinnamomum zeylanicum* against virulence gene expression inhibition of *Pseudomonas aeruginosa* and *Staphylococcus aureus* that are important in burn wounds. With attention to burn wound infections in hospitals and mortality increase in patients, it is necessary to design nanodressing. Clinical isolates were confirmed by biochemical and microbiological tests. DNA of isolates was extracted and PCR used to confirm the *alp* gene of *P. aeruginosa* and *Pv* gene of *S. aureus*. Polyurethane nanofiber and cinnamon polymers were used to prepare the scaffold under the electrospinning process. Infrared spectroscopy, electron microscopy, and mechanical tensile tests were used to confirm the scaffolds. The susceptibility testing and minimum inhibitory concentration of polyurethane-cinnamon nanofiber scaffold were determined against *P. aeruginosa* and *S. aureus*. For confirmation of polyurethane-cinnamon nanofiber scaffold were used the cytotoxicity test (MTT), FTIR, mechanical tensile test, and a scanning electron microscope. The expression of virulence genes was investigated using the real-time RT-PCR technique. The results of the susceptibility testing indicated that *P. aeruginosa* and *S. aureus* were susceptible to polyurethane-cinnamon nanofiber scaffold. The MTT, FTIR, mechanical tensile test, and SEM confirmed the different features of the polyurethane-cinnamon nanofiber scaffold. Results of real-time PCR demonstrated that the expression levels of *p–v* and *alp* genes after treatment decreased, respectively, 2.71- and 1.06-fold. Results indicated that the electrospun polyurethane-cinnamon nanofiber scaffold for the first time could inhibit both important pathogens of the hospital and the expression of the virulence genes. Considering the susceptibility of *P. aeruginosa* and *S. aureus* to and its inhibitory effect on *an alp* and *p–v* genes, this system could probably be a candidate in wound dressing for commercial purposes to burn healing and infection inhibition.

Keywords Polyurethane-cinnamon · Nanofiber · Scaffold

Extended author information available on the last page of the article

Published online: 11 January 2020

Introduction

According to the Center for Disease Control (CDC) reports, *Pseudomonas aeruginosa* is the second cause of hospital pneumonia in the USA and a third common cause of urinary tract infections in hospital and the seventh agent in hospital bacteremia, and in Europe reported as a third bacterium infection in ICU [1]. In recent years, despite the emergence of various types of antibiotics with broad-spectrum activity against *Pseudomonas aeruginosa*, this organism has caused severe infections in burns due to the acquisition of antibiotic resistance [2]. Antibiotic resistance is rising rapidly, and this trend has been proven in various treatment centers in the world as well as Iran [3].

P. aeruginosa contains multiple virulence factors, including exotoxins, adhesions, proteases, and has a high resistance to most common antibiotics [4]. This microorganism can infect bed, bed rails, sink burn and often acts as an opportunistic pathogen from recurrent infections in hospitalized patients [5].

Virulence factors include exoenzyme A, exotoxins S, U, T and Y, elastase, fimbriae, flagellum, lipopolysaccharide and pyocyanin that play a role in antibiotic resistance and microorganism pathogenesis [6]. Alkaline protease is one of the important virulence factors of this bacterium, which results in the decomposition of cytokines such as interleukin 2 and leukocytes. Drug resistance is emerging as a problem all over the world, and widespread use of antibiotics is likely to be the main cause of resistance in *P. aeruginosa* strains [7].

P. aeruginosa has an inherent and acquired resistance to most antimicrobial agents. Resistance to *P. aeruginosa* occurs through several mechanisms including β-lactamase production, efflux pumps, and outer membrane changes [8]. Therefore, it is necessary to find new therapeutic methods for inhibiting these microorganisms and reducing the costs of using antibiotics and preventing the dissemination of drug resistance genes and reducing mortality.

Staphylococcus aureus is a normal flora of the nose, the skin and digestive tract of some people. *S. aureus* is one of the five common causes of nosocomial infections, especially postsurgical ulcers. Each year, 500,000 people in hospitals of the USA are infected with *S. aureus* [9]. This bacterium has a spectrum of virulence factors. One of the important virulence factors in this bacterium is leukocidin (*p–v* toxin), which is common in all strains of MRSA *S. aureus* and also plays a role in the spread of cutaneous infections due to the elimination of the leukocytes [10].

Medicinal herbs are important drugs because of the general tendency of the community (fewer side effects, less environmental pollution), and widespread distribution [11].

The *Cinnamomum zeylanicum* belongs to the family Lauraceae, and the cinnamon genus contains 250 species. Cinnamon is a traditional herbal medicine that is native for Sri Lanka and is now widely grown in China, India, Indonesia, Ceylon, and Australia. Cinnamon has many uses that serve as a flavoring and preservative in food and desserts, in cosmetics, medical supplies, and medications. In the food industry, cinnamon is an ideal for antibacterial activity, anticancer,

antioxidant, and hypercholesterolemia, as well as an excellent source of minerals that is guaranteed by adding essential oils to edible food safety [12].

Cinnamon contains amidone, mucilage, tannin, a colored material, calcium oxalate, sugar, manito, essential oil and resin [13]. The essential oil of cinnamon is 1% in the skin of the plant, and its distillation is achieved with water. This essential oil is bright yellow when it is fresh, but over time, due to its oxidation, it turns yellow and then yellow to red. The bulk of this essential oil is cedar aldehyde. Also, it contains 4% of phenols, especially eugenols with Flanders, safrol, and furfurol. Eugenol in dentistry is used as an anesthetic and antimicrobial agent for teeth and gums and also helps to reduce blood glucose in diabetics [14].

Electrospinning is the simplest method for the preparation of fibrous scaffolds for use in tissue engineering [15]. Production of very thin fibers, easy functionalization for different uses, higher mechanical features and ease of processing are the most important advantages of the electrospinning technique [16]. The small diameters of fibers cause the high surface area than volume ratio or mass ratio, which makes them suitable candidates for an extensive range of usages [17].

Various polymers including natural polymers, synthetic polymers [18], and a copolymer comprising a natural and synthetic polymer have been used in electrospun fibers [19]. Scaffolds have many applications such as tissue engineering [20], wound dressing [21], drug delivery [22], and vascular grafts [23]. This study for the first time investigated the antibacterial properties of nanofiber scaffolds of polyurethane-*Cinnamomum zeylanicum* against major burn wound pathogens.

Therefore, this study aimed to investigate the properties of Nanofiber Scaffolds of Polyurethane-*Cinnamomum zeylanicum* against *the alp* gene of *P. aeruginosa* and *p–v* gene of *S. aureus*.

Methods

Bacterial strains

The clinical isolates *P. aeruginosa* and *S. aureus* which, respectively, contain the *alp* and *p–v* genes were identified and confirmed by phenotypic tests and PCR techniques.

Antimicrobial susceptibility test

The disk diffusion susceptibility test was used for antimicrobial susceptibility [24]. The test was performed by using a bacterial inoculum with a concentration of 0.5 Mc Farland on the surface of Mueller–Hinton agar medium. Twelve antibiotic disks were placed on the medium surfaces and incubated for 24 h at 37 °C. The growth inhibition zones around the antibiotic disks were measured. Then, the results were interpreted as susceptible, intermediate and resistance using the criteria considered by the Clinical and Laboratory Standards Institute (CLSI) [25].

The antibiotic disks used for *S. aureus* were carbenicillin (100 µg) ciprofloxacin (5 µg), oxacillin (30 µg), gentamicin (30 µg), bacitracin (10 U) and vancomycin (10 µg), and disks for *P. aeruginosa* were ciprofloxacin (5 µg), ceftazidime (30 µg), piperacillin (100 µg), amikacin (30 µg) and polymyxin B (50 µg).

Determination of MIC

In the broth macro dilution method, an overnight culture of *P. aeruginosa* was prepared in Pseudomonas agar. A stock of 0.5 McFarland was prepared as a control for turbidity comparison. Colonies of *P. aeruginosa* were inoculated in the physiology serum and prepared the 0.5 McFarland. The optical density for the bacterial suspension was measured. Absorption of bacterial suspension with a spectrophotometer at a wavelength of 625 nm was equal to 0.08–0.13, which indicated bacterial count approximately 1.5×10^8 CFU/mL. In this study was used polyurethane-nanoparticle nanofibers solution for studying disk diffusion and MIC.

In 8 tubes, 2 mL of Mueller–Hinton broth medium and 10 µL suspension of *P. aeruginosa* were added. To study the elasticity nanofibers scaffold, 0.5 µL of polymer solution with cinnamon essential oil with a concentration of 10 mg/mL was added to the first tube. Then we're prepared dilutions 0.5, 1, 1.5, 2, 2.5, and 5 µL. One reaction was considered as the negative control that only contained 2 mL of culture medium without bacteria and another as a positive control without a solution of polymer and cinnamon essential oil. The reaction tubes were incubated for 18–24 h at 37 °C. The incubated tubes were cultured on Mueller–Hinton agar and then incubated for 18–24 h at 37 °C. The first tube that inhibits bacterial growth was considered as MIC.

DNA extraction

The Promega kit (Ltd. Seoul, South Korea) was used for the extraction of DNA. Briefly, 1 mL of overnight bacterial culture was added to 1.5 mL microtubes. The microtubes containing bacteria were centrifuged at 15,000 g for 2 min, and the supernatant was discarded. In the next step, 600 µL of the nucleic acid lysis buffer was added to the bacterial pellet (the pipetting performed gently) and incubated at 80 °C for 5 min and then transferred to the room temperature. 3 µL of RNase solution was added and microtubes mixed gently by inversion. The microtubes were incubated at 37 °C for 15–60 min and then transferred to the room temperature. The amount of 200 µL of protein precipitation solution was added to the microtubes, and they were shaken to mix the protein precipitation solution. Microtubes were incubated on ice for 5 min. At this stage, the microtubes were centrifuged at 13,000–16,000 g for 3 min. The supernatant containing DNA was transferred to a 1.5 mL sterile microtube containing 600 µL of isopropanol and shaken slowly. At this stage, the microtubes were centrifuged at 13,000–16,000 g for 2 min. Then, the supernatant solution was slowly removed and 600 mL of 70% ethanol added to microtubes and centrifuged at 13,000–16,000 g for 2 min. The microtubes were dried at room temperature for 15 min. About 100 µL of rehydration solution was added to the microtubes and

incubated at 65 °C for 1 h. The DNA purified was stored at 4 °C. To the quantitative and qualitative analyses of DNA extracted, respectively, *Nano Drop*™ spectrophotometer and 0.8% agarose gel were used.

Design of primers

The primers were designed and then blasted on the NCBI Web site in a BlastN program, and the suitable sequence of primers was selected. The bacterial primers used in this study were related to the *alp* gene of *P. aeruginosa* (with amplicon 191 bp) and PV gene of *S. aureus* (with amplicon 194 bp).

PCR

For detection of isolates containing genes was carried out PCR. The total volume of the PCR for *alp* and *p–v* genes was 25 µL; each reaction consists of 1 µL of template DNA (20 pg), 12.5 µL of Master Mix (1×), (Pishgam), 1 µL of each forward and reverse primers (10 µM) (Pishgam) and 9.5 µL of distilled water (DW). The PCR was performed in a Thermocycler (Eppendorf vapo protect) through the following conditions: a primary denaturation step at 95 °C for 5 min; 35 cycles at 95 °C for 30 s, 60 °C at 45 s and 72 °C at 40 s; and 5 min, final extension at 72 °C. The PCR products were visualized on a 1% (w/v) gel agarose containing SYBR® Safe (Qiagen). DNA ladder 100 bp (Fermentase) was used.

Extraction of cinnamon essential oil

The extraction of cinnamon essential oil was performed using the Clevenger apparatus according to the method recommended in the British Pharmacopoeia for 3 h. For this aim, 50 g of the cinnamon samples was weighed and powdered using electrical grinders. The powder of cinnamon was poured into a 1-liter balloon and 50 mL of distilled water added to it. The essential oil was collected in tubes. The aqueous phase which has a specific boundary with essential oil was removed, and the pure essential oil was transferred into a glass container with a tight lid to prevent air penetration and was kept in the refrigerator to prevent oxidation [26].

The building of the scaffold

The construction of a scaffold consisted of two steps to make solutions and setting up of an electrical apparatus, which were as follows.

About 0.6 g of polyurethane polymer (up 51) was weighed using a digital scale and poured into a 25-mL volumetric flask. Then, 1.5 mL of dimethylformamide (DMF) and 4.5 mL of tetrahydrofuran (THF) were added. Then, it was placed overnight on the Stuart Hotplate stirrer with 300 rpm at temperature 70 °C. After dissolving the polymer in the solvent, 600 µL of the 10% cinnamon extract was dissolved in a ready solution. The solution was aspirated into the syringe. Then, the polyurethane solution was aspirated into two syringes equally. The special connectors were connected to syringes. A medium collector of electrospinning drive was used. It should be noted that the control

scaffold was not contained cinnamon extract. After placing the syringes into the electrospinning device, the program was adjusted as follows: nozzle angle: 45-degree, nozzle speed: 100 mm/min, drum speed: 240 rpm, syringe: 10 mL, needle: 10 mm, distance: 220 mm, flow rate (Debi): 0.3 mL/h, high voltage: 15 kV, timer: infinitely. The electrospinning time was at least 6 h, and the optimum time was selected depending on the thickness of the scaffolding.

Fourier Transform infrared spectroscopy

In this test, which is based on Fourier transform infrared spectroscopy (FTIS), the IR beam passes through the sample, and a part of it is absorbed by the sample and another part passes through the sample. The spectrum obtained shows the absorption and transmission that create a molecular "fingerprint" of a material that is unique for each sample.

This test was used to ensure the presence of cinnamon in the scaffold. For scaffold containing cinnamon and control, this test was carried out at Amir Kabir University, Tehran, Iran.

Mechanical tensile test

This test was performed to examine the tensile strength, to ensure that the scaffold made was suitable. The device used in this research was SANTAM, which measured at a speed of 50 mm/min and with an applied force equal to 0.5 kN (Fig. 2). At first, three pieces of scaffolds were cut in the size of 1×4 cm and used from a frame to keep the scaffolding fixed and prevent unwanted stretchiness. The frame containing the sample was attached to the two clamps of the device [27]. The settings of the device are abstracted in Table 5.

Scanning electron microscope (SEM)

Using the SEM, the morphology and topography of the scaffold are visible. The scaffolds were studied using an electron microscope located at the Amir Kabir University Faculty of Materials. The method to prepare the scaffold for SEM in the non-cellular modes was as cutting 1 cm.

Determination of surface hydrophilicity

The electrospun scaffold*s* were two groups: (a) the electrospun polyurethane plus cinnamon extract, and (b) electrospun polyurethane. The angle of contact with water was measured before and after placing the scaffolds in the plasma.

Plasma-coated electrospun scaffolds

To provide a suitable wettability (or hydrophilicity) surface for attaching the cell to the scaffold, they were placed for 6 min in a plasma device. The settings of the plasma device were as follows: frequency: 40 kHz, time: 6 min, power: 30 W, gas (oxygen): 99.99%, and gas pressure: 0.4 mbar.

Punching and sterilize the scaffolds

Regarding the type of test to be performed, scaffolds were punched at a diameter of 1 cm. To sterilize the scaffolds, after removing the foil from them, the UV device was used; and each side of the scaffold was placed in the device for 20 min.

Seeding the fibroblasts on the scaffolds to cell proliferation

After cells counting, the rate of the target cell carefully transferred onto the wet scaffold surface in a 24-well plate. Scaffolds were placed at room temperature for 5 min and then gently inserted into the incubator carefully. Every 15 min, a very small amount of DMEM medium containing FBS was added to the scaffolds. After 2 h, the remaining of the medium was slowly added to each well.

MTT assay

Human fibroblast cells were seeded on the fibrous scaffold with a diameter of 1 cm, which consisted of two test groups (polyurethane + extract and polyurethane alone) and a control group that was without scaffold and repeated 3 times. On certain days (1, 3, 7, and 14), OD was measured at 570 nm. The MTT stock was diluted in a high glucose medium (concentration of 5 mg/mL) and kept it in the dark place. The seeded cells on the scaffold were removed and were washed with PBS, and then on the cells, the DMEM without FBS medium containing the 10% was added (In 24-well microplate, 500 µL was added in each well) and incubated at 37 °C for 2 h. Then, the MTT was removed and added 500 µL of DMSO and pipetting was performed to dissolve the formazan particles. The microplates were gently shaken and measured at 570 nm using the ELISA reader spectrophotometer.

RNA extraction

To extract RNA using the Jena bioscience GmbH, Germany, *P. aeruginosa* and *S. aureus* were cultured in BHI medium and incubated for 24 h at 37 °C. The 0.5-McFarland suspension of bacteria was prepared. Then, the contents of each tube were centrifuged after incubation for 5 min at 10,000 rpm. The supernatant was discarded and stored at − 20 °C to the extraction of RNA. 1 mL of RNX-Plus solution was added to each of the sediment and mixed the tubes gently and then placed at room temperature for 10 min. About 200 µL of chloroform was added, and the tubes were shaken gently to mix completely and placed at room temperature for 2–3 min

and at a temperature of 4 °C for 15 min and then were centrifuged at 9000 rpm. After centrifugation, the organic phase (the chloroform in the lower part), the middle phase (containing the protein) and the supernatant phase (consisted of a colorless liquid nucleic acid) were formed. The RNA remained in the liquid phase, this phase was carefully and without interruption, the middle phase was separated and transferred to a clean tube of 1.5 mL (the volume of the liquid phase was about 60% of the volume of the *tetrazolium* solution used). About 500 µL isopropyl alcohol was added to the RNA precipitate and the tubes were shaken slowly and the samples placed at room temperature for 10 min. Then, tubes were centrifuged at 4 °C for 15 min at the speed of 9000 rpm. The supernatant was discarded and RNA precipitate kept. The RNA precipitate was washed using 75% cold ethanol. This step was performed twice, and after each washing step, the centrifuge was performed at 4 °C with a speed of 6000 rpm for 5 min. The white precipitate obtained was placed at room temperature for evaporation of the alcohol, and finally, 100 µL of distilled water treated with DEPC was added. The tubes were stored at − 70 °C.

cDNA synthesis

The first step of RT-PCR was to convert RNA to cDNA. In this study, at first 9 µL of RNA was mixed with 1 µL of random hexamer primer, and with adding nuclease-free water, the reaction final volume was 10 µL and then vortexed for 30 s and then, for denaturation, the RNA tubes were placed at 75 °C for 5 min and was placed on ice. At the next stage, RT-enzyme buffer and dNTP were added to tubes containing RT-Transverse Transcriptase Enzyme Reaction (RT). Finally, the volume of the final reaction was 15 µL with nuclease-free water. The reaction tubes were placed in a PCR check device for 80 min. To inactive, the exonuclease activity of the reverse transcriptase enzyme, the inactivation phase of the reverse transcriptase enzyme was performed at 60 °C for 5 min. The cDNA synthesis reaction consists of 1.5 µL of DEPC water, 1.5 µL of dNTP (µmol), 1µL of poly N primer (50 µmol), 0.5 µL of RT (200 unit/µL), 1.5 µL of RT buffer (10×) and 9 µL of RNA(100 ng/µL) in the final volume of 15 µL.

Real-time RT-PCR

Real-time RT-PCR was performed using the Syber Green stain and the ready RT-PCR mixture (Takara Co.). The total reaction real-time RT-PCR volume (25 µL) included 12.5 µL (1×) of qPCR SYBR Green Master Mix, 1 µL (1 µg) of DNA template, 1 µL (10 µm) of both reverse and forward primers and 9.5 µL of distilled water. Then, 1 µL of cDNA of each sample was added to each well. After preparing the reaction mixture, reactions were placed in Applied Biosystem (AB) device and real-time RT-PCR incubation was performed in 1 cycle of primary denaturation (95 °C for 10 min), 40 cycles in secondary denaturation (95 °C for 15 s) and primer annealing (53 °C for 60 s).

Statistical analysis

All data were obtained at least with the triplicate, averaged and expressed as mean ± standard deviation (SD). The experiment was repeated twice. We used one-way analysis of variance (ANOVA) for statistical analysis. Differences were considered statistically significant at $p \leq 0.05$.

Results

Results of disk diffusion susceptibility test

The results showed that *P. aeruginosa* was resistant to carbenicillin disk but susceptible to ciprofloxacin, gentamycin, imipenem, meropenem, polymyxin B, neomycin, streptomycin and tobramycin (Table 1). As shown in Table 2, *S. aureus* was susceptible to all disks used but only resistant to Bacitracin.

Results of MIC test

The MIC was evaluated using broth microdilution according to CLSI 2016. Results showed that the MIC of *P. aeruginosa* and *S. aureus* was 0.04 and 0.02 µg/mL, respectively.

Table 1 Results of disk diffusion susceptibility testing of *P. aeruginosa* strains to antibiotics used

Antibiotic	Unit	Inhibition zone (mm)	Susceptible (S) Intermediate (I) Resistance (R)
Carbenicillin	100 µg	12	R
Ciprofloxacin	5 µg	40	S
Gentamycin	10 µg	22	S
Imipenem	10 µg	30	S
Kanamycin	30 µg	11	R
Meropenem	10 µg	39	S
Neomycin	30 µg	18	S
Polymyxin B	300 u/IE	18	S
Streptomycin	10 µg	9	S
Tobramycin	10 µg	20	S

Table 2 Results of disk diffusion susceptibility testing *S. aureus* to antibiotics

Antibiotic	Unit	Inhibition zone (mm)	Susceptible (S) Intermediate (I) Resistance (R)
Gentamycin	10 µg	22	S
Linezolid	30 µg	35	S
Trimethoprim	10 µg	34	S
Oxaciline	30 µg	20	S
Polymyxin B	300 u/IE	18	S
Vancomycin	5 µg	24	S
Bacitracin	10 U	8	R
Novobiocin	10 µg	20	S

Amplification results of *alp* gene from *P. aeruginosa* and *p–v* gene of *S. aureus*

As shown in Fig. 1, the band amplified by the replication of the *alp* gene of *P. aeruginosa* was an amplicon with a size of 191 bp. Also, the amplification of the *p–v* gene of *S. aureus* indicated a DNA amplicon with a size of 194 bp.

Measurement of the diameter inhibition zone of the electrospun scaffold with cinnamon extract at a concentration of 10% to *P. aeruginosa* and *S. aureus* was reported to be 39 and 41 mm, respectively (Fig. 1).

The results show that the nanofiber scaffold containing the extract has an appropriate bactericidal activity against two hospital important pathogens.

The results showed that the nanofiber scaffold showed more antibacterial activity than antibiotics in the test.

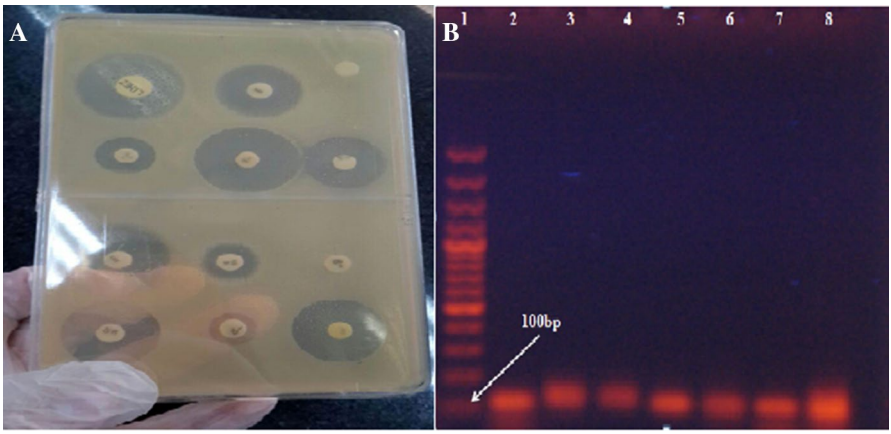

Fig. 1 **a** Image of the diameter of the inhibition zone of the *electrospun* scaffold with cinnamon extract at a concentration of 10% are related to the *P. aeruginosa* and *S. aureus.* **b** Gel electrophoresis of PCR products of *alp* and *p–v* genes. The well M: DNA Ladder Mix100 bp 1. The wells 2, 3, 4 PCR products of the *p–v* gene of *S. aureus* (Amplicon size 194 bp). The wells 5, 6, 7 PCR products of *alp* gene of *P. aeruginosa* (Amplicon size 191 bp)

The samples are positive for *p–v* gene *S. aureus. Well* M: DNA Ladder 100 bp, ells 1–4: The samples positive for *the p–v* gene. PCR products of *the alp* gene of *P. aeruginosa*. Wells 5–8 *alp gene.*

The results of FTIR spectroscopy test

This test was based on infrared spectroscopy. The results of the infrared beam transmitted from the control and the electrospun scaffolds with polyurethane-cinnamon are shown in Fig. 5.

Electrospun scaffold with polyurethane-cinnamon like the control scaffold has a sharp peak in the site of carboxyl (1700–1730 cm^{-1}), which confirms the presence of polyurethane-cinnamon in the electrospun scaffold. The main characteristic of polyurethane is the presence of a ketonic absorption bond (C=O) in the absorption range (1700–1730 cm^{-1}). According to the results, the highest peak obtained in the scaffold of polyurethane plus cinnamon extract was in the site of 1732 cm^{-1} which is related to the strong bonding of carboxylic acid, showing the effect of cinnamon on polyurethane. The peak of the extract group, after adding to the scaffold, showed an 85% increase in adsorption (reduction of transmittance) and in a scaffold without extract showed 78% (Fig. 2).

This test was performed to determine the mechanical properties (tensile strength) of the scaffold, and to ensure that the scaffold obtained in terms of strength was suitable for the treatment of bacterial infections. In this strain–stress curve, the threshold rate of strain in the elasticity region of the scaffold of polyurethane nanofibrils alone and polyurethane with cinnamon essential oil was 3.94% and 0.096%, respectively, indicating a linear change in the scaffold formation, the slope of which was the elasticity modulus Fig. 2. According to the results, the scaffolding was extended with increasing force and not broken down under low forces.

As shown in Table 3, the scaffold contained the electrospun cinnamon extract compared to the control scaffold had a less Young's modulus. Also, adding the cinnamon extract reduced the maximum stress, but did not change in the maximum strain.

In this test, a standard dumbbell model is drawn from two sides at a constant speed. To a torn rusty sample, the mechanical properties of the material are determined by recording the instantaneous moment to the moment of change in sample length.

The results of the hydrophilicity, the angle of contact and scanning electron microscope (SEM)

This test was conducted to ensure that the plasma-scaffolds were hydrophilic. The presence of plasma causing increases the oxygen functional group on the surface and increases the contact angle (Fig. 3).

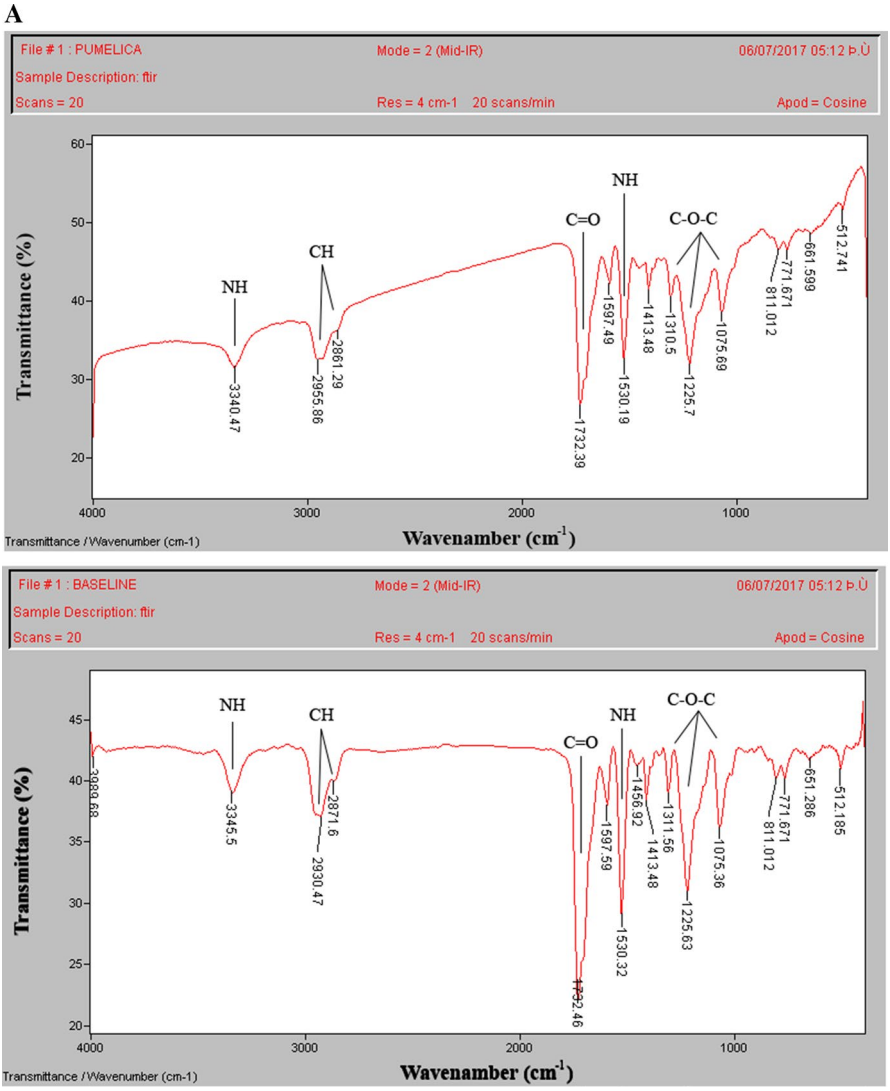

Fig. 2 **a** The result of the FTIR test. The ketonic bond, which is a strong bond, visible in the chart at intervals of 1700–1730 cm^{-1}). **b** Results of mechanical tensile test. **b.1** Control scaffold, **b.2** electrospun polyurethane-cinnamon scaffold

The scaffolds were examined by a scanning electron microscope. The results of the study showed that there is no difference in the fiber diameter of the two groups. Image J software was used to measure the diameter of the fibers.

The *T* test was also used to measure the significant difference in the diameter of fibers.

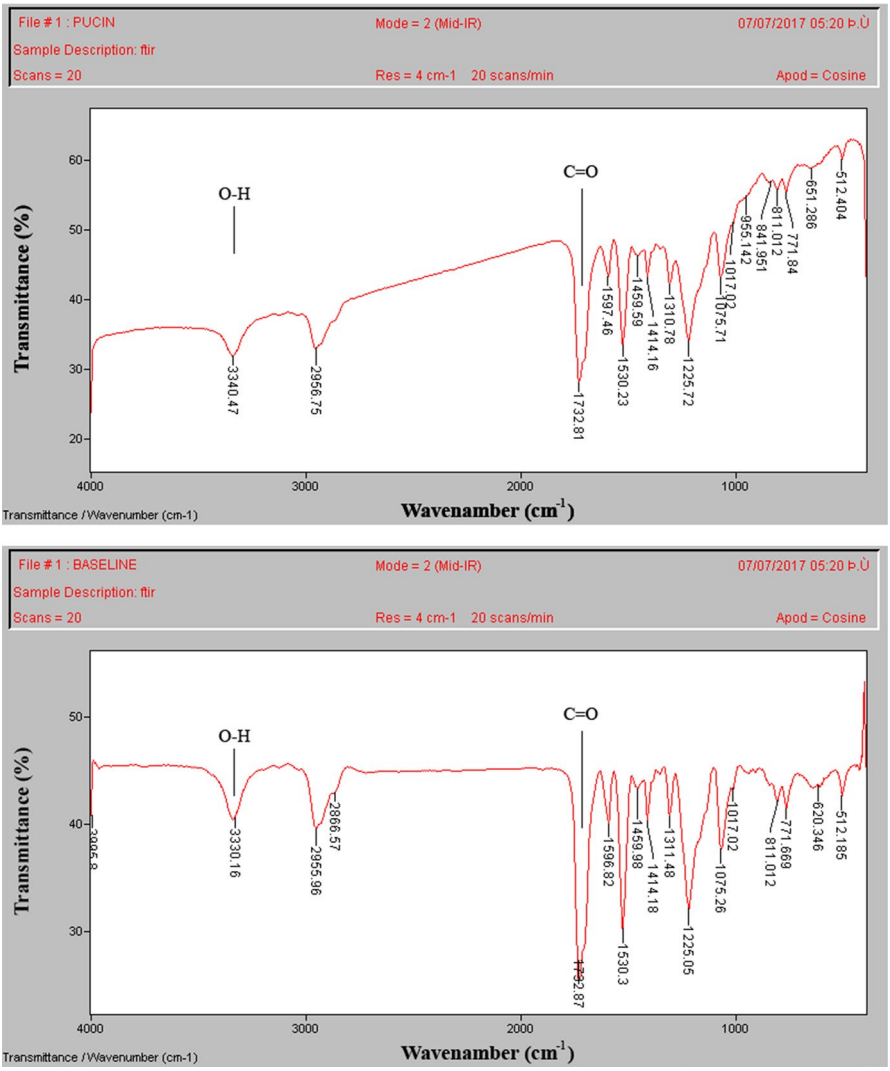

Fig. 2 (continued)

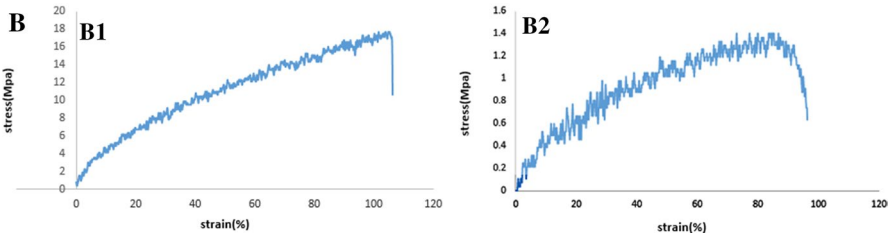

Fig. 2 (continued)

Table 3 Results obtained from Young's modulus

Sample	Width (mm)	Thickness (mm)	Gage length (mm)	Young's modulus
Polyurethane	10	0.066	20	0.752 ± 0.02
Polyurethane + Cinnamon	10	0.035	20	0.096 ± 0.01

There was no significant difference in scaffolding in polyurethane with polyurethane and cinnamon groups (Tables 4, 5).

The results showed that there was no difference in the diameter of the fibers of the two groups, and the difference was not significant (p value = 0.208) (Fig. 3).

The results indicated that there is no difference in the fiber diameter of the two groups. Compared to control scaffolds, nanofibers are not much different.

Results of the cytotoxicity test and real-time RT-PCR

The survival of human fibroblast cells was investigated in 14 days and the presence of a medium without FBS DMEM in a 24-well plate. Survival rates on days 1, 3, 7 and 14 were measured (Fig. 4). According to the results, the scaffold containing cinnamon exhibited more adhesion and growth and cell proliferation than both control and polyurethane scaffolds. The maximum absorption was observed on the 14th day in the scaffold containing cinnamon.

The Livak method was used to investigate the changes in the expression of the studied genes using the data achieved from real-time PCR. This method was calculated based on the formula $\Delta\Delta$Ct than to the changes in target gene expression compared to the control sample. As shown in the following, the expression level of the *p–v* gene after treatment showed a 2.71 times decrease ($2^{-}\Delta\Delta\mathrm{Ct} = 2^{0.09} = 1.06$).

The *alp* and *p–v* genes were evaluated using the real-time RT-PCR technique. The melting chart of each sample was drawn after the completion of the process by measuring the fluorescence variations at various temperatures. In this way, the device changed the temperature of the samples at specified intervals and simultaneously the fluorescence curve was plotted in terms of temperature. The peaks observed in the Tm chart show the product (Figs. 4, 5).

As the results show, the scaffold containing cinnamon exhibits more adhesion and growth and cell proliferation than the control scaffold and polyurethane scaffold. The maximum absorption was observed on the 14th day in cinnamon scaffolds.

MIC test results

The results of the MIC of *P. aeruginosa* and *S. aureus* were evaluated using broth microdilution according to CLSI 2016. MIC results according to serial

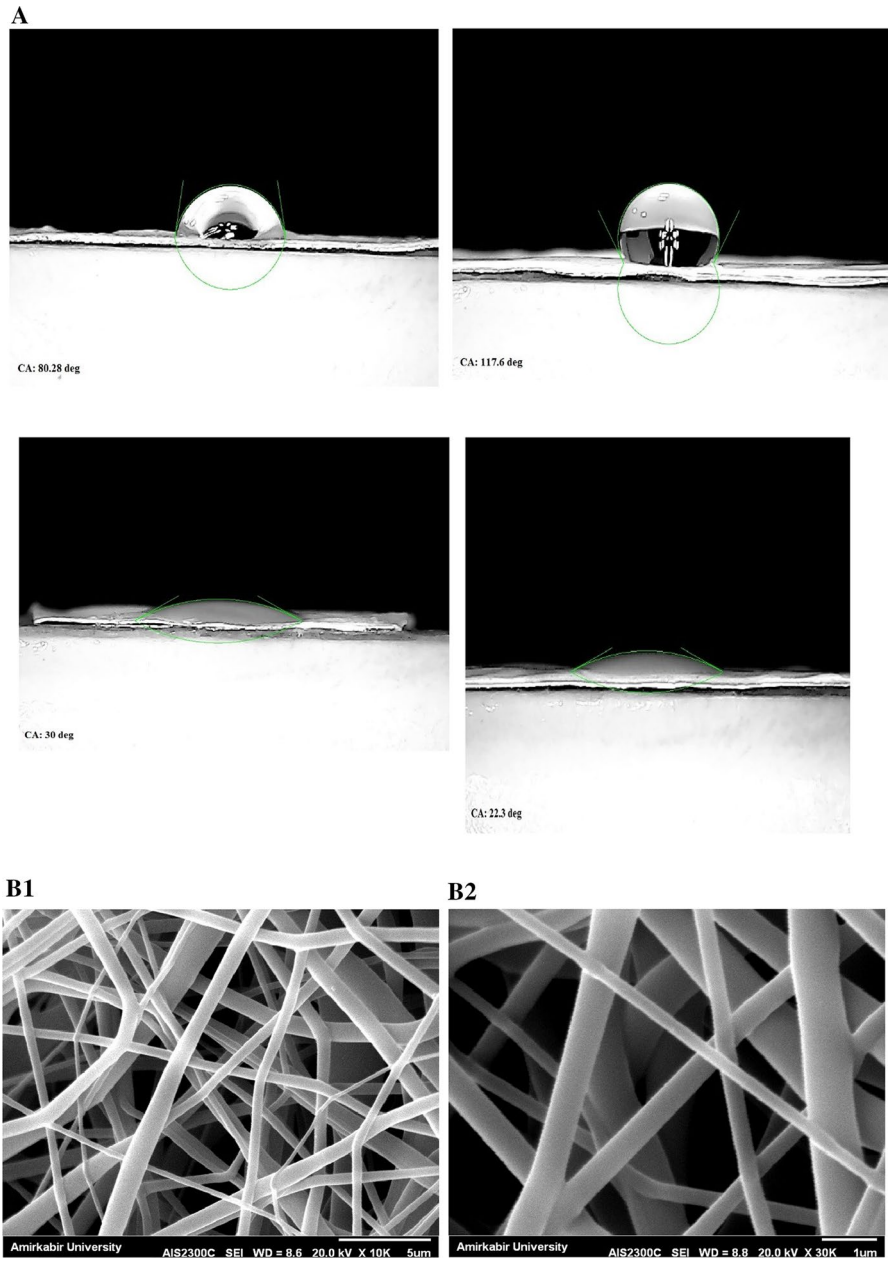

Fig. 3 **a** The results of the hydrophilicity and the angle of contact of the scaffolds before and after coating with plasma. **b** Scanning electron microscope (SEM). **b.1** Control scaffold with the magnification of 5 microns. **b.2** Electrospun cinnamon and polyurethane scaffold with a magnification of 1 micron

Table 4 The measurement of fibers diameter using Image J software

Group	Average	SD
PU	0.5892	0.470
PU + Cin	0.910 ± 0.267	0.699 ± 0.241

Table 5 The settings of the mechanical tensile device

Sample	Width (mm)	Thickness (mm)	Gage length (mm)	Young's modulus
Polyurethane	10	0.066	20	0.752 ± 0.02
Polyurethane + Cinnamon	10	0.035	20	0.096 ± 0.01

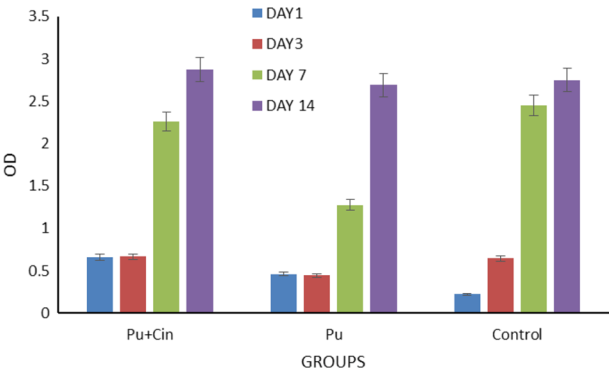

Fig. 4 **a** The results of the MTT test on days (1, 3, 7 and 14) for both electrospun and control scaffolds. **a** Diagram of product melting. **A** Melting graph of reference gene (*gyr* A), **B** the melting plot of the *alp* gene that was in the range of 85–86 °C, **C** melting graph of reference gene (*gyr* A), **D** the melting plot of the *p–v* gene that was in the range of 85–85.75 °C

dilution showed that the MIC for *P. aeruginosa* and *S. aureus* was 0.04 µg/mL and 0.02 µg/mL, respectively.

Discussion

Overuse and self-administration of antibiotics is now one of the major causes of the spread of antibiotic resistance. Overdose of broad-spectrum beta-lactam drugs and prolonged hospitalization of patients cause the spread of high-resistance bacteria. Increased resistance of Pseudomonas aeruginosa to antibiotics raises the issue of infection control in burn patients.

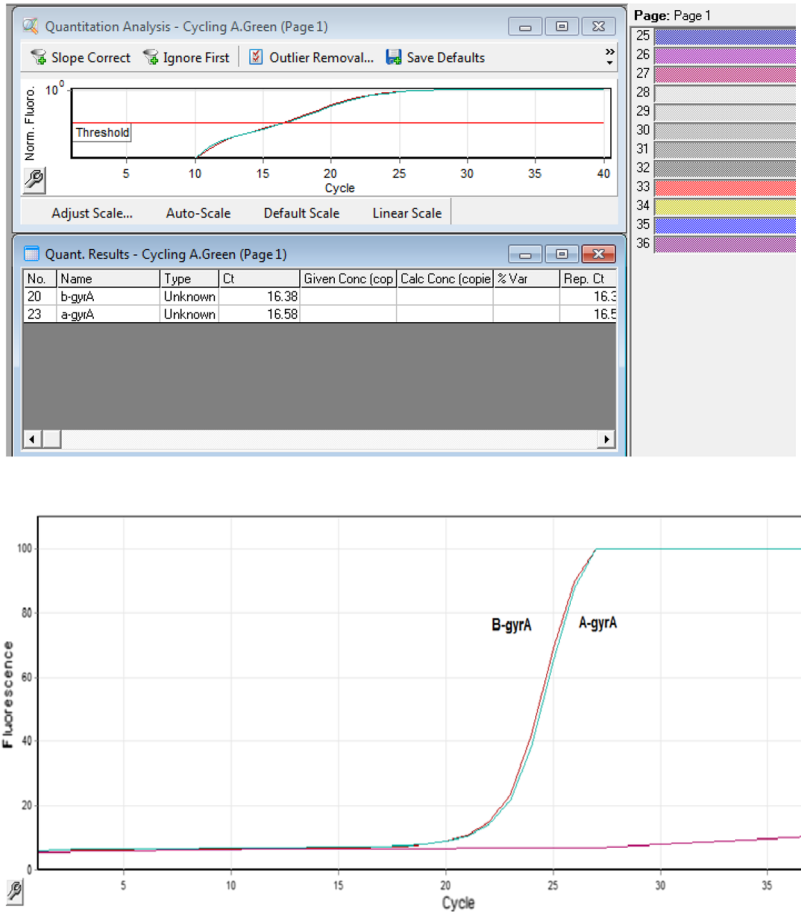

Fig. 5 A graph related to the results of the real-time RT-PCR using the step one software v2.3 software, which automatically generated after each test. The results of the study revealed the expression of the *p–v* and *alp* genes in a standard strain of *P. aeruginosa* in the presence of polyurethane-cinnamon nanofibrous scaffold. This graph shows the progress of the reaction in exponential mode. The red line at the bottom of the threshold graph is 0.016 °C. Threshold is the reaction where the insertion point is marked with a red arrow (color figure online)

Nowadays, MDR and XDR strains are a worldwide problem. The incidence of the mentioned strains is increasing due to the selective pressure of inappropriate antibiotics and the increasing dose of the drug.

P. aeruginosa is an opportunistic pathogen and an important agent of nosocomial infections. The increasing resistance to antimicrobial drugs, especially beta-lactam drugs, causes severe infections like pneumonia, septicemia, and skin infections (in people with burn), [28]. It is also the third leading cause of hospital infection and the second most common cause of infection in burn wounds [29].

S. aureus is one of the most important acquired agents in the hospital, especially surgical wounds by medical instruments. Methicillin-resistant strains, in addition to

affecting the people in the hospital, can be acquired from the community and colonized in the host's body. This bacterium has many virulence factors that can protect it in the host body [10]. One of the important virulence factors is leukocidin (toxin *p–v*), which is present in all strains of MRSA *S. aureu*s and also plays an important role in the dissemination of cutaneous infection due to the destruction of leukocytes [10].

Due to the therapeutic problems and drug resistance and the high mortality rate owing to *P. aeruginosa* and *S. aureus*, an effective and efficient method is necessary for immediate treatment. Plants were the first compounds that examined by humans as drugs and showed good effects over time. Traditional medicine has been used for thousands of years ago for therapeutic purposes. The use of essential oils and medicinal plant extracts has been widely used. Recently, the use of nanofibers and electrospun fibers has attracted much attention. Among these, the use of electrospun scaffolds with plant extracts as new therapeutic agents against diseases and microbial infections.

A study was conducted in China, in which the antimicrobial effect of cinnamon (CEO) on *S. aureus* was investigated. Cinnamon essential oil with MIC = 1 µg/mL indicated the antibacterial activity against this bacterium. MBC for *S. aureus* was 2 g/mL [30].

Also, electrospinning is a process in which nanofibers are produced from solutions or mixtures of polymers in the presence of electrons, which help to regenerate the skin due to the structure of the scaffolds. The construction of nanofibers increases the possibility of reaching scaffolds that can strongly adhere to the natural texture of humans on a nanometer scale [31].

Extract or essential oil of the cinnamon contains the active ingredient cinnamaldehyde with antibacterial property; the electrospinning of this material along with elastic polymers can provide a scaffold which with its gradual release, prevents from the possibility of bacterial infections and, on the other hand, minimizes the patient's need for a dressing change. Electrospun scaffolds have nanoscale fibers which, due to the increase in surface to volume, make it possible to release excess extracts or essential oils during treatment [31]. Therefore, the purpose of this study was to design and investigate the inhibitory properties of Polyurethane-*Cinnamomum zeylanicum* nanofiber scaffolds against *the alp* gene of *P. aeruginosa* and *p–v* gene of *S. aureus* using Real-time RT-PCR technique.

Luis Dias et al. to ensure the presence of *prp* in their electrospun scaffolds used by spectrometric FTIR, and they observed that the scaffolds containing the freeze-dried *prp*, as well as the scaffold containing the *prp* coating, both at the site of the amides 1 and 2 have the peaks [32]. We also examined the control scaffolds and scaffold electrospun containing polyurethane-cinnamon and in the range of 1700–1730 cm^{-1} in the test scaffold, and a ketonic transplant was observed, which was a reason for the presence of polyurethane-cinnamon in the scaffold.

Luis Dias et al. measured their scaffolds using the tensile mechanical test and concluded that adding *prp* to scaffolds would reduce Young's modulus and the strain and tension slightly. It can be inferred from these results that after adding the *prp*,

scaffolds become drier; as a result it is easier to torn [32]. About the results obtained from the mechanical tensile test, we also concluded that the addition of polyurethane-cinnamon to the scaffold during the electrospinning caused a slight decrease in tension and also a decrease in Young's modulus, but the scaffold strain did not change.

An electron microscope can be used to examine the surface properties on the scaffold. An ideal scaffold should have even and no beaded fibers to provide a suitable environment for growth, cellular communication and differentiation [33]. Chunxia Gao et al., using an SEM, investigated the microstructure of scaffolds randomly and ensured the absence of beads in its structure [34]. In this study, the test and control scaffolds were also examined using an SEM and ensured the randomization of the fibers, the absence of beads as well as the size of the fibers in the scaffold. Also, regarding the diameter of the test and control scaffolds can be said that the cinnamon due to diluting the dimethyl formaldehyde (solvent), which results in thickening of the polyurethane fibers of the scaffolds containing electrospun cinnamon.

Soleimani et al. using MTT assay concluded that their cells were found on nanofibrous scaffolds without producing any toxicity [35]. In the study conducted by Byong-Taek et al., on scaffold containing *prp* using the MTT test it was observed that the use of *prp* in the scaffold probably due to its growth factors caused the ideal cell density and consequently the cellular viability [27]. As the mentioned study, we also investigated the cytotoxicity of test and control scaffolds over 14 days, and we observed that compared to the standard culture plate, a combination scaffold not only has no negative effect on cell proliferation or any toxicity, but they increased the cell proliferation than control scaffold.

One of the innovations of the present study is that for the first time the effect of polyurethane-cinnamon scaffold on *the alp* gene of *P. aeruginosa* and *p–v* gene of *S. aureus* was investigated.

The Livak method was used to investigate the changes in the expression of the studied genes using the data achieved from real-time RT-PCR. The results showed that the expression levels of the *p–v* gene after treatment 2.71 times decrease. Also, the expression level of *the alp* gene after treatment showed the 1.06 times decrease [36–40].

Our study suggested that the effect of polyurethane-cinnamon scaffolds as a dressing for infectious wounds in a burned rat model in vivo checked. A nanofiber polyurethane-cinnamon scaffold could be checked as a supplement to the treatment of infected wounds in dressing the infectious wounds.

One of the innovations of the present study is that the effect of polyurethane-cinnamon scaffold on *alp Pseudomonas aeruginosa* and *p–v Staphylococcus aureus* genes was first investigated.

In this study, a quantitative technique such as real-time RT-PCR was used to evaluate the expression of *alp* and *p–v* genes of *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

In this study, the Livak method was used to study the expression changes of the studied genes using real-time RT-PCR data. The use of the Livak method to study

the expression changes of the genes studied is another aspect of this study compared to previous studies.

Due to the therapeutic problems, drug resistance and high mortality caused by *Pseudomonas aeruginosa*, effective and efficient treatment is needed to treat this bacterium immediately. Traditional medicine relying on natural products has an important role in the treatment and prevention of this bacterium.

All methods were carried out by relevant guidelines and regulations.

All experimental protocols were approved by the BMSU licensing committee.

For this research, consent was obtained from all subjects.

Conclusion

Based on the acquired results, polyurethane-cinnamon electrified has the best results in most of the tests performed in this study, and it can be said that polyurethane-cinnamon electrified has the potential to be employed for antibacterial purposes by applying wound dressings to the medical community. However, as electroporated polyurethane-cinnamon has been less effective in some tests such as relative expression, further research is needed.

In this study, the effect of essential oil of cinnamon against *P. aeruginosa* and *S. aureus* has been proved. On the other hand, since many types of research on electrospun cinnamon have not performed such as antimicrobial, and gene inhibition against *alp* in *P. aeruginosa* and *p–v* in *Staphylococcus aureus*.

One of the disadvantages of this study is that recent nano scaffold has not been investigated in animal and human models. Pathological studies are needed to confirm wound healing and pathogen inhibition in this in vivo state.

Nanodegradable can be offered to the medical community for the treatment of burn wound infections and increased wound healing and to reduce antibiotic use.

Polyurethane-cinnamon scaffolds can be offered to the medical community for the treatment of burn wound infections and increased wound healing and to reduce antibiotic use.

Acknowledgements We would like to thank our colleagues at BMSU, for their help.

Authors' contributions DE conceived the project and designed the study. HH performed experiments. DE analyzed data and wrote the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by the authors.

Availability of data and materials The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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