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Preparation of wound dressings using levan from *Lactobacillus gasseri* and polyvinyl alcohol polymer

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Abstract

Levan had been extracted, purified, and characterized from *Lactobacillus gasseri*. Levan characterization had been carried out using "thin-layer chromatography (TLC)", 1HNuclear-MagneticsResonances (HNMR), and Fouriere-transforms infrared spectroscopies (FTIR). Characterization demonstrated that pure levan is a homopolysaccharide with just fructose and atypical for carbohydrate bond area of 868 cm-1. The maximum water solubility index (WSI) percentage was 90% at a conc. of 100 mg/5 ml, while the highest water holding capacity value had been 252 at a conc. of 50 mg/5 ml. The wound dressings were prepared using purified levan from *L.gasseri* and its blend with polyvinyl alcohol (PVA) polymer, and the polymer add-on percentage and the reduction of *Pseudomonas aeruginosa* growth were calculated. The proportion of polymer applied to cotton gauze has been shown that the polymer add-on for three types of polymers include levan, PVA, and the mixture of levan and PVA were (177.00, 81.50, and 290.50) % respectively. The reduction of *P. aeruginosa* growth by the polymers-coated gauze investigated that there was no bacterial growth in levan-PVA blend coated cotton gauze and the bacterial reduction percentage was 100% compared to levan coated cotton gauze with 52.00% for burn isolate and 66.66% for wound isolate, while the bacterial reduction of PVA coated cotton gauze was 43.00% for burn isolate and 55.55% for wound isolate**.** On the other hand, the present study included evaluating the effectiveness of the prepared levan-PVA cotton gauze bandage through its use in dressing rabbits that were injured and infected with *P.aeruginosa*. The present results showed that the prepared levan-PVA cotton gauze bandage led to faster wound healing, without harmful effects, and enhanced wound healing during the first week compared to traditional cotton gauze alone and antibiotic cotton gauze.

Keywords: Levan, PVA, Anti-bacterial, Wound dressing

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Introduction

Levan is indeed a polysaccharide made up of (β2→6) - linked fructofuranosyl residues that have been branched through (β2→1) linkages (Xu et al., 2016). The technological applications and biological activities of different levans are determined by the length of the polymeric chain, and the

amount of branching. Because of their enormous molecular weight, and mechanical and rheological properties, microbial levans would often be performed (Veerapandian et al., 2020). Levan-based thin films for wounded tissue repair have been the subject of some of the most. promising medical application investigations (Ağçeli and Cihangir, 2020a). Phosphonate levan and chitosan have been used to create a hybrid multilayer film prepared with various technologies for these levan films (Costa *et al*., 2013), and using a levan-based film to create wound dressings (Tabernero *et al*., 2019). Levan is involved in metalloproteinase activation, which is an important stage in the repair of burned or mechanically injured tissues (Sturzoiu *et al.*, 2011; Ragab *et al*., 2020). Levan was discovered to be an appropriate polymer for laser-based technology, permitting films with a levan gradient and an oxidized levan (Sima *et al*., 2012). Cell proliferation was highest on oxidized levan, with significant development at the transition between the native and oxidized levan (Sima *et al*., 2011; Axente *et al*., 2014). PVA was a synthetic polymer, it possesses a variety of remarkable features, including non-toxicity, strong chemical stability, biocompatibility, and excellent film-forming ability, and is frequently blended with natural polymer to improve natural polymer's mechanical performance (He *et al*., 2017). Due to its strong polarity and solubility, PVA as a hydrogel may be used as a component for cornea replacement, access to vascular and equivalent of epidermal skin, furthermore, the PVA hydrogel in three-dimensional structure gives an effective microenvironment as maintaining a continuous moist environment, cells need to proliferate in the region of wound and recovery efficiency (Negishi *et al*., 2014; Morales-Hurtado *et al*., 2015; Lin *et al*., 2019). PVA had long been used in dressings and therpy of a wound, artificial organs, drug delivery systems, and contact lenses, furthermore, hydrogel PVA had insufficient flexibility, a hard membrane, and extremely hydrophilic qualities, it couldn't be used alone as a wound dressing polymeric membrane, other polymers might have been bent with PVA to provide a wound dressing that heals faster (Kamoun *et al*., 2017; Lin *et al*., 2019). Polymeric wound dressings which depend on hydrogels assisted pressure ulcer patients' healing by promoting quicker epithelialization, and as a result, the number of wounds that were healed with hydrogel dressings increased in comparison to individuals who received conventional gauze dressings (Sood *et al*., 2014). This study aimed at the preparation wound dressing using purified levan and its blend, and treatment of wound infection *in vivo* using prepared wound dressing.

Material & Method

Microorganisms

"Lactobacillus gasseri"

A healthy woman's vaginal swab was used to isolate *Lactobacillus gasseri*, it has been afterward cultivated anaerobically for (24–48 hours) in MRS medium at 37°C. The isolate has been identified using the Vitek 2 technique as well as cultural, microscopic, and biochemical tests.

Pseudomonas aeruginosa

Burns and wounds were used to isolate clinical *P. aeruginosa* isolates. The isolates were identified using the Vitek2 approach together with cultural, microscopic, and biochemical assays. The four isolates with the highest levels of virulence factors and antibiotic resistance were selected for this investigation. All isolates possessed its virulence factors(biofilm production, swarming, hemolysin, and pyocyanin) and antibiotic sensitivity tested (data not shown).

Precipitation and Purification of Levan

The cultured *L. gasseri* had been centrifuged at 10,000 rounds per minute / 10 minutes after being incubated for 24 hours, levan had been precipitated from the supernatant by adding 2 vol. of cold 100% ethanol. After being dried at (40-45)C^{o}, the levan's dry weight was calculated. To completely remove light M.Wt. particles, Levan polymers had been rained and dialyzed (cut-off at 14 kDa) for a minimum 2 days, Levan had been dissolved in water, and the supernatant had been separated from a water-insoluble component with centrifuged (10,000 rpm / 10 mins), after repeating the process with 2 vol. of 75% ethanol after the material precipitated in two vol. of cold 100% ethanol, the levan had been dissolved in water and dialyzed after being centrifuged at 10,000 rpm/10 minutes (cut-off 14 kDa) (Haddar *et al*., 2021). Using a spectrophotometer, the O.D. had been measured at 400 nm, the equation described by González- Garcinuño *et al*. (2017) was used to determine the levan concentration:

“y = 0.1645x −0.035”

Where x was levan conc. given in mg/ml and “y” was ABs at 400 nm.

After that, purified levan had been dried at (40-45^{o}C) and stored for future investigation.

Characterization of Purified Levan

Analyses of Levan (TLC)

TLC had been accomplished on a plate that is coated in silica gel (60–120 mash), 0.01gm levan purified from selected isolate. Levan would have hydrolyses (5% HCl v/v), had overheated 1 hr inside (water bath100^{o}C), in 1 ml of 1% ethanol, equal weights (0.01gm) of G, S, and F (glucose, sucrose, and fructose) would hve dissolved (as standard). Following that, capillary tubes had located 10 l of hydrolyzed levan and other sugar solutions equally spaced apart from the TLC plate's bottom margin, around 2 cm, a sealed jar containing mobile phase, It has a composition of, cyano methane, EtOAc, PrOH, EtOH, & H_2O_2 (8:2:5:2:10), had just been filled with the plate. The plate was then permitted to diffuse through silica gel, after some time, a plate was taken out of the container and permit it to air-dry at 25C^{0}, whenever diffusion had reached around 7.5 cm. The dried plate had been sprayed in the face with a particular reagent consisting of 0.3% (w/v) β-naphthol & 5% (v/v) H_2SO_4 solution in EtOH,

and it had been placed in an oven for 5–10 minutes at 105°C. Levan constituents became visible as black patches (Kim, 2006), and their location and distance would have been determined, and Rf would have been calculated as investigated by Radhi *et al*. (2013):

$$Rf = \frac{\text{Distance moved by substance}}{\text{Distance moved by the solvent front}}$$

"Fourier-Transform Infrared Spectroscopy (FTIR) Analysis"

To determine how the functional groups of the levan had deposited, FTIR analysis had used. Departments of Chemistry/ Collage of Sciencee/ Mustansyrie University, Iraq, using an FTIR spectrophotometer to detect the FTIR spectrum to transmit data between wavenumbers of 4000 and 400cm-1.

H Nuclears Magnetics Resonances (HNMR) Spectroscopies

Iran, Tehran received levan powder by doing so, HNMR spectroscopy which was used to characterize the purified levan**.**

Water solubility index WSI

WSI means material's degrees of dissolvable in water. WSI of levan had been detected by using the method described by Domżał-Kędzia *et al*. (2019). To create a homogenous solution, 200, 150, 100, and 50 mg levan had thawed 5 ml deionized H_2O_2, vortexed per 40 mins /40°C. The sample had been centrifuged at 4000 rpm/ 10 min, and supernatantes had layed inside a petri-dish that was already weighed. This was done to acquire a dry solid weight. , using the following equation to determine WSI:

$$\textbf{WSI (\%)} = \frac{dry\ weight\ of\ solids\ in\ supernatant}{weight\ of\ dry\ sample} \times 100\%$$

Water-holding capacity

Quantity waters that can hold material had measured its WHC. WHC of levan had detected with the method of Domżał-Kędzia *et al*. (2019). Levan had been maintained at 40 °C for 10 mins after being dissolved in 10 ml Milli-Q water. 200, 150, 100, and 50 mg of levan had also been dissolved. The sample had then centrifuged for 30 minutes /14000 rpm, and the supernatant was discarded. The pellet has therefore dried by setting on pre-weighed filter paper. A precipitated sample weight has been noted. The following equation has been used to get the WHC percentage:

WHC (%) = $\frac{\textit{Total sample weight after water absorption}}{\textit{Total dry sample weight}} \times 100\%$

Preparation of wound dressing

Wound dressing was prepared using biopolymer levan 10% (purified from *L. gasseri* in other studies), polyvinyl alcohol (PVA) polymer 6% after dissolving it in distilled water at 90°C, and combination (1:1) of levan with PVA. Then the solutions were poured into a petri dish after 10 min of stirring, the cotton gauze with a diameter of 4cm was soaked with three groups of polymers separately to become the polymer-soaked medium and stored at 60°C for 48 h (Lin *et al*., 2019 with modification).

Calculating the polymer add-on percentage of prepared wound dressing

According to the method of Shanmugasundaram and Gowda (2011), after drying, a weight of samples was calculated then the polymer add-on percentage of prepared wound dressing was calculated using the following equation:

Polymer add-on (%) = $\left[\frac{(W_1\text{-}W_2)}{W_2}\right]\times 100$

Which W1 and W2 are the weights of coated and uncoated cotton gauze, respectively. For each cotton sample that had been coated with polymers, the polymer add-on % was determined independently.

Bacterial reduction of prepared wound dressing

The effect of prepared wounds dressing on two isolates of *Pseudomonas aeruginosa* growth (burn & wound resources) was identified by applying the given method by Venkatrajah *et al*. (2013):

Gauze samples prepared would have been transferred to a flask (250ml) and inoculated with 1 ml of bacterial isolates (compared with McFarland), approximately $1.5x10^8$ CFU/ml. After incubation at 37C° for 24h, 100ml sterilized distilled water had poured to flask, mix it for 1 min, then make a 10-fold serial dilution. Then transfer streaked on nutrient agar with 0.1 ml from every diluted then incubated for (24-48) hrs in 37 C°. After incubation No. of the colony had to be countered.

The below calculation processed which compute percentage of bacterial decrease (Venkatrajah *et al*., 2013):

$$R\ (\%) = \frac{(B\text{-}A)}{B}\times 100$$

Where A is the number of bacterial colonies number from cotton gauze coated and B is the bacterial colonies number from cotton gauze uncoated.

Treatment of wounds infections using a prepared wound dressing

The experiment included laboratory albino rabbits, left for 72 h before making wounds to adapt to the surrounding environment. Animal hair has been removed in the dorsal area (where the wounds are to be caused) using the hair remover, then sterilized the region using 70% ethyl alcohol and left for 24 h. to avoid any infections and for anesthetized used xylazine (5mg/kg) with ketamine (100mg/kg). By intramuscular injection and by sterile surgical blades of size 10 were created full-thickness skin wounds 3cm in diameter, as suggested by Odimegwu *et al*. (2008) & Golbui Daghdari *et al.* (2017) with modification. Rabbits had divided into 3 main groups (4 rabbits of each group) including:

1- The first group: which included rabbits were injured and infected with wound *P.aeruginosa* isolates and bandaged with the classical cotton gauze.

2- The second group: which included rabbits were injured and infected with wound *P.aeruginosa* isolates and bandaged with levan-PVA blend polymer cotton gauze.

3- The third group: which included rabbits were injured and infected with wound *P.aeruginosa* isolates and bandaged with the antibiotic cotton gauze.

After the wounds were made, observing the animal's behavior, and kinetic activity, and evaluating the wound region for inflammation, redness, pus, and any other signs, as well as wound healing duration and if a cicatrix remains after recovery, after confirmation of infection exposed the wounds of animals as mentioned in Jain *et al.* (2009). Following the infection, all groups were bandaged with cotton gauze as described above after being cleansed daily. Treatment of all groups was observed for 15 days (Mekkawy *et al*., 2017).

Results

Precipitation and Purifications of L. gasseri (Lb7)' levan

Levan had been precipitated and purified from *L.gasseri (Lb7)*, and Every step of the purification process involved an investigation into the levan conc.. Levan conc. There are concentrations at each stage of purification, 2.036, 6.474, 8.601, and 9.884 mg/ml, respectively, before precipitation, following precipitation, following dialysis, and following the last purification(Table-1). The yield of purified levan reached 16g/L.

Table 1.

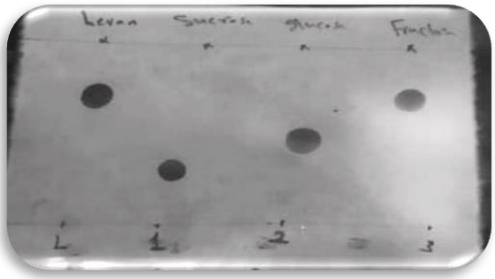
Concentration of levan at different precipitation and purification steps.

Steps	O.D	Levan concentration(mg/ml)
Before precipitation	0.37	2.0365
After precipitation	1.1	6.4742
After dialysis	1.45	8.6018
After last purification	1.661	9.8845

Characterization-techniques for Levan-Purified (L. gasseri (Lb7))

Analysis of Levan by (TLC)

TLC had applied which can identified simpler sugars of Levan that *L. gasseri* had purified. The Rf value of fructose was identical to extremely similar to acid hydrolyzed levan. Levan had an Rf of 0.66 As opposed to glucose, sucrose, and fructose, which displayed Rfs of 0.40, 0.66, and 0.60, respectively. This demonstrated that the fructose-based *L.gasseri* purified levan had been used (Figure 1).



L S G F

Figure 1.

TLC analysis (purified levan'*L.gasseri* (Lb7)

L: Levan; S: sucrose; G: Glucose; F: Fructse

FTIR analysis

Examining the FTIR-spectra of *L.gasseri* (Lb7) revealed the structural features of pure levan. The purified levan's peaks demonstrated that it is a polysaccharide type. (O-H) stretch-vibrations of

hydroxyl had reflected band 3275.24 cm^{-1}, whereas the (C-H) stretch-vibration had reflected with band 2935.76 cm^{-1} range. Stretching of the C=O boundary has been responsible for the band's 1635.69 cm^{-1}, region-band 1415.80 cm^{-1}-1257.63 cm^{-1} indicated stretch of an aromatic skeleton, &C-H bending. The vibration of C-O-H stretching is shown by band 1118.75cm^{-1}.

The prominent peaks 1010.73 cm^{-1}, which had a carbohydrate fingerprint, reflect C-O-C stretch-vibration and glycosidic bond within pyranose/furanose. Measurements 922.00 and 802.41 proved that bands 771.55cm^{-1}, which are characteristic of pyranose, are a component of the fructose unit, as well as a binding area for carbs, was at least 868.00cm-1 (detection of polysaccharides) (Table 2).

Table 2.

Position of a functional group of purified levan from *L. gasseri*(Lb7)

Position	Group
3275.24	O–H
2935.76	C–H
1635.69	C=O
1415.80	C-H bending
1257.63	C-H stretch
1118.75	C–O–H stretch
1010.73	Glycosidic bond

Analysis of 1H Nuclear magnetic resonance (1HNMR)

Additionally, 1HNMR-spectrum showed proton-chemical-shift-signal associated with fructose, which had been detected as levan's monomer (Figure 2). This signal had been identified as 4.68 p.p.m(OH-g), 4.75 p.p.m(OH-e), 4.62 p.p.m(OH-f), 3.79 p.p.m(H1-c), 3.99 p.p.m (H1-h), 3.63 p.p.m (2H-a), 3.71 p.p.m (H1-b), and 3.65 p.p.m (2H-d).

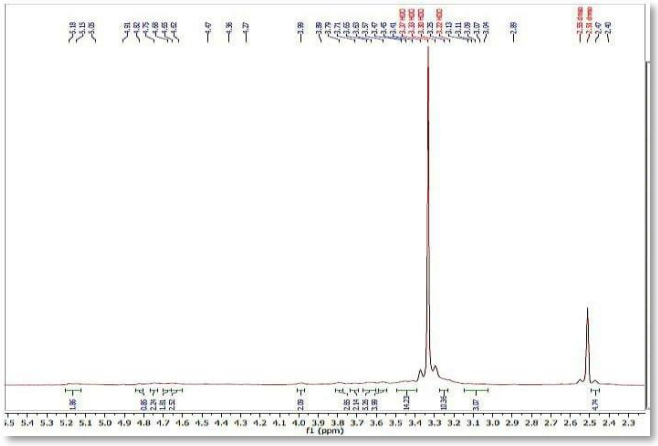


Figure 2.

1H Nuclear magnetic resonance (HNMR) of levan purified from *L.gasseri* (Lb7)

Water solubility index and water-holding capacity

The degree of a substance's solubility in water has been determined using WSI. The water quantity that a substance can maintain has known as its water-holding capacity (WHC). The outcomes are shown as the number of times that levan powder weight could be retained in the water. The WSI and WHC had been assessed at various concentrations of pure levan. WHC value ranged from 150 at conc. 200mg/5ml to 252 at conc. 50mg/5ml. The highest WSI percentage had been 90% at concentration 100mg/5ml, whereas the minimum WSI had been 79%at conc. 200mg/5 ml (Figure 3).

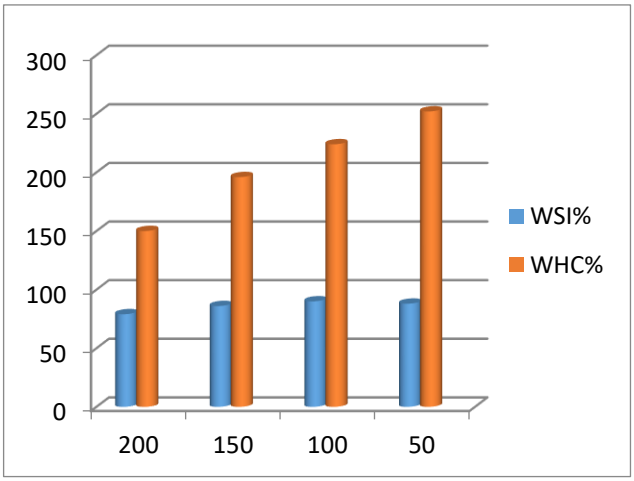


Figure 3.

Percent of (WSI) and (WHC) of *levan* purified from *L. gasseri* (Lb7).

Polymer add-on in cotton gauze

The proportion of polymer applied to cotton gauze has been shown in table (3). The percentages of polymer add-on for three types of polymers include levan, PVA and the mixture of levan, and PVA were (177.00, 81.50, and 290.50) % respectively. The presence of polymers was added to the prepared cotton gauze shown in Figure (4).

Table 3.

Polymer add-on in cotton gauze

Polymer add-on to cotton gauze	Un-coated cotton gauze weight(g)	coated cotton gauze weight(g)	%Polymer add-on
Levan	0.200	0.554	177.00
PVA	0.200	0.363	81.50
Levan + PVA	0.200	0.781	290.50

PVA: Polyvinyl alcohol

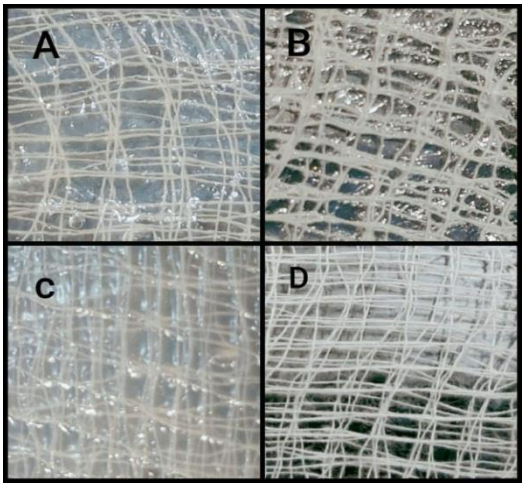


Figure 4.

Coated cotton gauze with biopolymer levan and its blend with PVA.

A: levan-coated gauze, B: PVA-coated gauze, C: mixed levan + PVA-coated gauze, D: uncoated cotton gauze.

Bacterial reduction of prepared wound dressing

Polymers' antimicrobial properties coated gauze had been evaluated for two *P. aeruginosa* isolates, one from a wound and the other from a burn, and the bacterial reduction % was determined. The results investigated that there was no bacterial growth in levan + PVA blend coated cotton gauze and the bacterial reduction percentage was 100%, compared to levan coated cotton gauze with 52.00% of bacterial reduction for an isolate from burn and 66.66% for wound isolate, while the bacterial reduction of PVA coated cotton gauze was 43.00% for burn isolates and 55.55% for wound isolate (Figure 5).

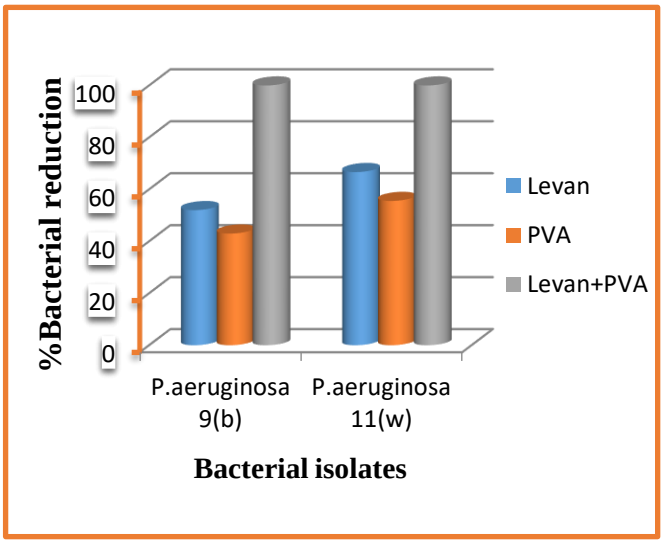


Figure 5.

Reduction of *P. aeruginosa* in prepared wound dressing

Treatment of wounds infections using prepared wound-dressing

Adult rabbits were selected and separated randomly into groups to study dermal wound healing, which is known as a difficult process that requires appropriate harmony between various components of skin to allow the repair of injured tissues and restoration of normal skin functioning. The control group had infected with *P.aeruginosa* and bandaged with traditional cotton gauze to compare visual healing times with the treatment groups. Antibiotic cotton gauze was chosen for its antibacterial capabilities in eliminating infection from bacteria that infect wounds. This study had been evaluated the efficacy of the newly bandaged levan-PVA blend polymer cotton gauze during the treatment of wound infections. During the evaluation study, levan-PVA blend polymer cotton gauze had discovered to exhibit a very promising antimicrobial activity against the wound-infecting isolate. Group I and group II were bandaged with classical cotton gauze and levan-PVA blend polymer cotton gauze respectively and group III was bandaged with antibiotic cotton gauze, when the wound creation and infected by *P.aeruginosa* , bandage of this group was started (Figure 6) , then lasted for 15 days. A wound in Group II was closure faster when compared with each group I and group III, which showed

the highest wound reduction (healing) throughout the experiment when compared to the others values measured. However, in the current *in vivo* wound-healing study, levan-PVA blend polymer cotton gauze promoted wound healing. The infected wounds of the classical and antibiotic cotton gauze-treated rabbit groups had not healed, and they also took longer to heal and were filled with pus at the infection sites. Animal deaths were not seen during the trial in any of the groups that were being looked at, and the hair had been grown faster and was thicker in the group treated II which bandaged with levan-PVA blend polymer cotton gauze after recovery on the 7th day. According to current study results, local use of levan-PVA blended polymer cotton gauze leads to considerably quicker wound healing, without adverse effects, and enhanced wound healing as compared to traditional antibiotic cotton gauze and classical cotton gauze alone.

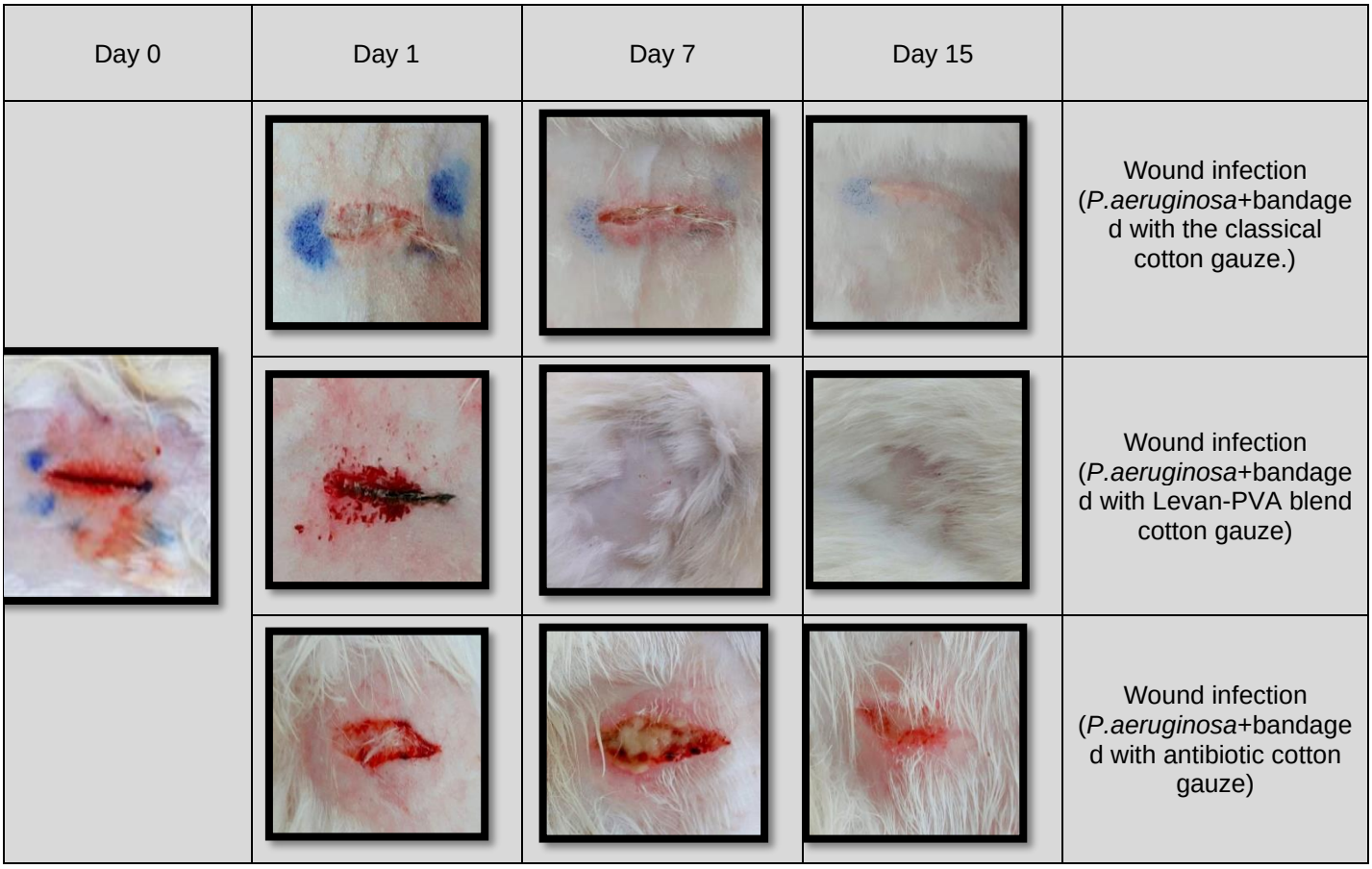


Figure 6.

The wound healing process in rabbit's model infected by *P.aeruginosa* using levan-PVA wound dressing.

Discussion

Haddar *et al.* (2021) revealed that ethanol precipitation and dialysis were employed for effective extraction and purification, resulting in 22g/l EPS (*B. mojavensis)*. Bouallegue *et al*. (2020) uncovered that the strain, growth circumstances, carbon supply, beginning pH, media, Temp., I.P., and inoculum size all had an impact on how much EPS bacteria generated.

Levan from *Bacillus siamensis*, which completely hydrolyzed into fructose (Thakham *et al*., 2020), similarly, according to Al-Qaysi *et al*., (2021). The sole component of levan generated from *P. agglomerans-ZMR7* which was also subjected to hydrolysis acid & TLC was fructose.

Gamal *et al*. (2020) used FTIR-analysis to investigate the levan generated extracted from *Enterococcus faecalis* and found pyranose sugars had significant for the absorptions at 1070.390 and 1271.82 cm^{-1}, according to Korany *et al*. (2021), FTIR analysis revealed the existence of CH2-OH&CH-OH stretching vibration bands of levan-isolated *Pseudomonas fluorescens*, 1010 cm^{-1} (844 and 891 cm^{-1}).

According to Korany *et al*.,(2021) proton chemical shift signals in 1HNMR spectra of *P. fluorescens* would have been found at 4.11 ppm (H-3), 4.02 p.p.m. (H-4), 3.86 p.p.m. (H-5), 3.82 ppm (H-6,a), 3.69 ppm (H-1,a), 3.59 ppm (H1,b), and 3.48 p.p.m. (H-6,b), δ4.11 p.p.m. (H-3), 4.02 p.p.m. Fructo-furanoside linkage(2→6), and protons between 3.2 and 4.2 ppm in the 1 HNMR spectrums indicate the presence of the levan type (Açeli *et al*., 2020b). Numerous applications, including tissue-engineered, regenerative medicine, and nano/micro drug delivery systems all show great potential for levan-based nanosized systems. Due to its large M.Wt and extensive hydrogen bonding had been formed self-assembled spheroids in water (Cinan *et al*., 2021).

The melting point of levan was about 225°C, based on Bostan *et al*. (2014), indicating that this polymer had excellent thermal stability. Likewise, Mantovan *et al*. (2018) demonstrated levan that was purified from *Bacillus subtilis natto* CCT 7712 displayed multiple thermal decomposition peaks between 200 and 225°C.

Pei *et al*. (2020a) demonstrated that purified levan from Bacillus megaterium PFY-147, had higher WHC (231.29%) and WSI (97.34%). WSI & WHC for standard levan from *Erwinia herbicola* (Sigma-Aldrich) were estimated to be 86.30 % and 1527.20 %, respectively (Domżał-Kędzia *et al*., 2019). Saravan & Shetty (2016) found that EPS purified from *Leuconostoc lactis* KC117496 had WSI and WHC 14.20% and 117.00%. The result investigated by Venkatrajah *et al*. (2013) showed that the surfaces of prepared gauze, chitosan-sodium-alginate-coated-gauze, and chitosan-sodium&calcium alginate coated gauze have all been covered with sodium and calcium alginate, the percentage of polymer add-on indicated36.8, 42.4, and 33.4, respectively.

Shanmugasundaram and Gowda, (2011) investigated that the sodium alginate polymer and chitosan were blended and coated with the padding mangle on the gauze structure and that the calcium plus sodium alginate polymer had coated by bamboo fabric with a 121.66 % add-on percentage (based on the weight of the material).

Li *et al*. (2008) revealed that adding biopolymer dextran to the culture media inhibited the cell growth of mesenchymal stromal cells. That had been due to dextran's high viscosity. After 24 hours of

seeding cells on the hydrogel, Lin *et al*. (2019) revealed that more cells adhered to the hydrogels with greater dextran concentrations, suggesting that these hydrogels offer a favorable environment for cell growth.

Shanmugasundaram and Gowda (2011) have determined that the bamboo samples with polymer coatings and medication loading showed exceptional antibacterial effectiveness against *Proteus* spp. and *S. aureus*. As a result, bamboo gauze textiles with polymer coatings and medication loading are ideal for surgical bandaging and wound healing. Kumar *et al*. (2013) obtained similar observations when reported for antibacterial activity of the β-Chitin Hydrogel/Nano ZnO composite bandages proven versus (*S. aureus&E.coli*). Demirci *et al*. (2020) suggested that the unique properties of Halomonas' levan hydrogels, such as their excellent biocompatibility, made them suitable candidates for application as wound dressing materials. The current study was similar to Bi *et al* (2020), which had been investigated that dressings have effect on *in vivo* wound healing, that bandage-treated.

Healing of Wounds was faster in general than the group of control, there were no notable significances in the way the wound healed within the PLA&AS/PLA/PVA groups. These findings revealed that PLA materials' had maintained appropriate fiber shape as a wound dressing, bio-compatibility, and a porous structure facilitated wound healing. The biodegradable fibrous scaffolds enhanced wound healing as a consequence, the outstanding cell conductivity due from fibers was capable of directing tissue regeneration from a tissue-engineering standpoint, all of this aided in the healing of wound and regeneration of skin (Zahedi *et al*., 2010). The healing of wound therapy in hydrogel using PVA/St+10% Cs+nZnO hydrogel exhibited the greatest antibacterial activity and healing of the wound on the 14th day, according to Baghaie *et al.* (2017).

The healing of wound potential of chitosan- films containing chondroitin-4-Sulphate+gelatin+ zinc oxide nanoparticles was investigated in animal models with full-thickness incisions, according to Cahú et al. (2017). After six days, the wound had shrunk significantly (14–35 %) compared with a control group.

Venkatrajah *et al*. (2013) inspected the performance of functional cotton gauze, finding that cotton gauze covered by chitosan-sodium alginate-calcium alginate polymer has a greater wound healing efficacy. The most frequent form of biopolymers are polysaccharides and engaged in a wide range of biological activities. They've also been used as a biomaterial for the healing of wound applications, including acceleration of epidermal tissue activities and skin regeneration (Kumar *et al*., 2018). The most extensively utilized biopolymers include clitosan, chitin, cellulose, pullulan, chitosan, starch, alginate, hyaluronic acid, and collagen which have hemostasis, antibacterial, anti-inflammatory, proliferative, and tissue adhesion qualities and play important role in the healing of the wound (Sahana and Rekha, 2018). Hemostasis, the formation of a fibrin clot, which functions as a shield versus outside influences, also crucial for moisture retention, is the initial step of healing of the wound (Velnar *et al.*, 2009).

Rasool and Islam, (2019) revealed that stimuli sensitive (CS) and (PVP) would have acquired hydrogel characteristics, which might be used in applications for wound healing. In cultured fibroblasts, elastin biopolymers boosted chemotactic activity, fibroblast proliferation, and collagenase expression (Devalliere *et al*., 2017).

PVA Functionalized is a viable choice for bandage material due to its non-carcinogenicity, non-toxicity, excellent biocompatibility, and swelling in aqueous solutions with a high degree (Kamoun *et al*., 2017). As possible cross-linking agents for PVA, any chemical that can react with O-H group can be employed (Shiu *et al.*, 2018). PVA had already been documented by esterification, carbonate and etherification, with the resulting compounds having distinctive characteristics and uses in a wide range (Alves *et al*., 2012).

Barthasarathy *et al*. (2021) investigated PVA nanofibers' mechanical qualities that could be improved and employed in smart bandages in the healing of a wound. Xia *et al*. (2021) reported the *in vivo* examination of blended dextran and PVA (DEX/PVA) on wound healing, the presence of DEX/PVA in medium quickens, indeed enhances wound closure, and DEX/PVA dressings demonstrated outstanding wound healing characteristics after 16th days when compared to saline treatments.

The Heparin-PVA and Au nanocomposite hydrogel bandages had been used as a dressing material in *in vivo* trials and have a strong bactericidal activity, a remarkable hydrophilic design, biocompatibility, and healing of wound ability, making it a feasible solution for burn injuries treatment (Zhang and Zhao, 2021).

Conclusion

In conclusion, purified levan could be coating cotton gauze with good polymer add-on percentage and reduction of bacterial growth, and showed an increase in polymer add-on percentage and reduction of bacterial growth after being mixed with PVA polymer. Levan - PVA blend coated cotton gauze bandage had the potential to be a typical material for wound dressing for its ability to enhance faster wound healing of rabbits without adverse effects.

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Conflict of Interest

No conflicts of interest were declared by the authors.

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Ethics Statement

Approved by local committee.

Authors' contributions

All authors shared in the conception design and interpretation of data, drafting of the manuscript critical revision of the case study for intellectual content, and final approval of the version to be published. All authors read and approved the final manuscript.

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