

RESEARCH ARTICLE

Production of Wound Dressing with Nano Fibers contain Bassorin/ Ofloxacin for Improvement Burn Wound

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ABSTRACT

Gum Tragacanth (GT) obtained from *Astragalus Gossypinus* is one of the most widely used natural gums which has found applications in many zone because of its appealing features such as biodegradability, nonpoisonous nature, natural accessibility, moisture absorption and creating a network of Hydrocolloid. It also has maintenance and delivery of drugs, higher resistance movement to bacterial attacks and lengthy shelf-life properties.

In present study, preparation nanofibers of 50 wt% Bassorin (extracted from Gum Tragacanth) has been mixed by 50 wt% Poly Ethylene Oxide and 0.01 wt% Ofloxacin (Ba/PEO/Ofx) for Electrospinning. Nanofibers coated on cotton gauze. The properties of Bassorin and produced nanofibers were examined via XRD, FTIR and SEM microscopy. The Antibacterial of nanofibers activity against *Staphylococcus aureus* as gram positive bacteria and *Escherichia coli* as a gram-negative bacteria also were studied. Nanofibers are able of absorbing wound's exocrine liquid easily due to their high specific area of nano fibers which 4 to 5% more than cotton gauzes without nanofibers. When it is conversion to gel by moisture sorption, the release of loaded Ofloxacin would be increased. The Antibacterial assay showed the cotton gauze coated with Ba/PEO/Ofx nanofibers could inhibit about 90% growth both bacterial strain on burn wound.

Also, the therapeutic effect of nano-bassorin in restoring superficial second-degree burns in rats showed an accelerated effect on wound healing. Based on the results of this study, it is possible to use cotton gauzes coated with bassorin nanofibers as a suitable candidate for the treatment of second-degree superficial burns.

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INTRODUCTION

Since old ages, diverse herbal resources have continually had specific significance and aids in the wound-healing procedure[1]. Gum Tragacanth (GT) with a history of use extending over some five thousand years is a dehydrated exudation attained from the stems and branches of Asiatic types of *Astragalus gossypinus* which contains two

main segments: water-soluble fractions namely Tragacanthin (low quantity of an Arabinogalactan) and water-swellable part is Bassorin. Tragacanthic acid and Bassorin are unsolvable in ethanol, and the other part Arabinogalactan is soluble in a combination of ethanol-water (7:3)[2]. The unique properties of GT such as easy to prepare, biodegradable, eco-friendly, inexpensive,

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natural available and also safe final resulted in the production of scaffolds from GT were used for many aspects of skin healing, drug carrier, and periodontal defect regeneration[3]. GT be formed of a linear 1,4 linked α -D-galacturonic acid backbone with three types of side chains: single β -D-xylopyranose, disaccharide units of 2-O- α -L-fucopyranosyl-D-xylopyranose and 2-O- β -D-galactopyranosyl-D-xylopyranose Fig. 1A [4] and the main structural of GT Fig. 1B. Bassrin structure extracted from GT shown in Fig. 1C[4].

The Gum Tragacanth is considered as generally accepted as safe at the 0.2–1.3% level in food materials in the USA since 1961. It is also consent to food adding in European Union and has the No. E413 in the list of adding confirmed by the Scientific Committee for Food of the European Co [5]. This biodegradable and biocompatible biopolymer is not allergenic, mutagenic, teratogenic and carcinogenic with no adverse toxicological effects in non-allergic people [5–7]. Some investigators have reported the application of Gum in different fields such as the green synthesis of silver nanoparticles [8], immobilizing agent in viral plaque assay [9], hydrogel membranes [10], dressing for healing of burn wounds [11], superlative absorbent hydrogel [12] and carrier for controlled release of drugs, etc

[13]. Khajavi and coworkers produced GT fibers by solution spinning method via alkaline treatment and they investigated the effects of spinning machine parameters on the mechanical properties of produced fibers [14,15].

A straightforward and efficient method of obtaining ultra-fine fibers is electrospinning technique with diameters within from micrometers to nanometers. Many properties can be seen from electrospun fibrous textiles such as high specific surface area and high porosity, with small pore size. Also, the especial uses of electrospun mats are wound dressing, tissue engineering and drug delivery [16–18]. It has been displayed chitosan biopolymer able to wound healing in human and also shown significant antibacterial activity against various type of bacteria [18–19].

Nowadays, various types of nanofibers and nanoparticles with different properties such as antibacterial, antifungal have been produced for use in biomedical fields. For this purpose, various antibacterial materials ranging from chitosan to drugs were used in nanofibers formulation[20]. In recent decades, antibiotics are known as an intense source of medicinal agents for infections treatment[21]. In a similar study, Ranjbar-Mohammadi et al. investigated PLGA/Gum

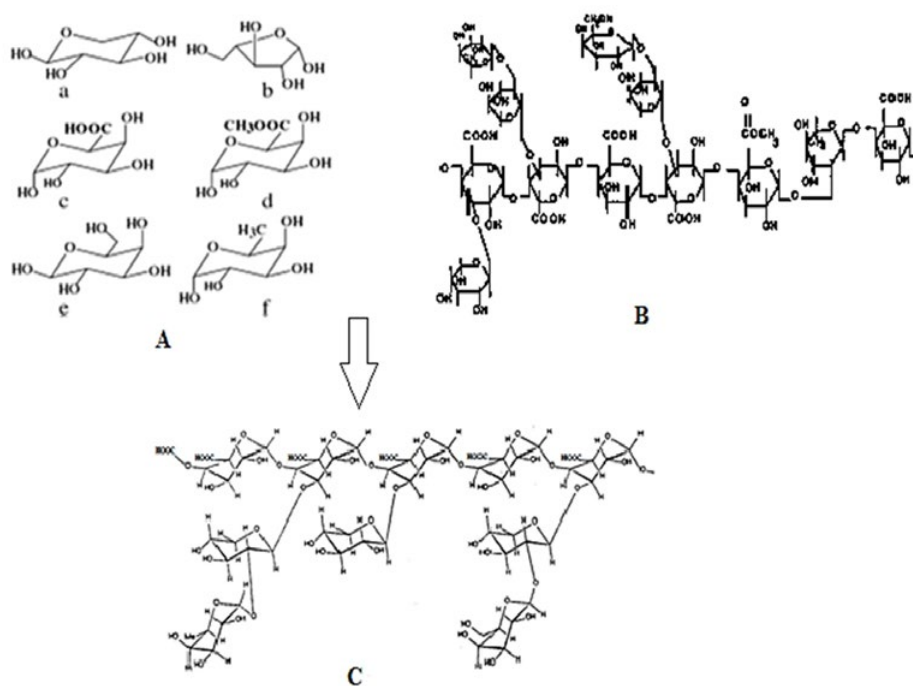


Fig. 1: (A) Main chemical building units of the GT) β -D-xylose L-arabinose,) α -D-galacturonic acid, d) α -D-1-galacturonic acid methyl ester) β -Dgalactose)1 α -L-1-fucose, (B) The main structural of GT, (C) Structure of Bassrin [4]

Tragacanth nanofibers containing Tetracycline for periodontal regeneration. Their results showed the produced nanofibers can release tetracycline effectively and inhibit bacterial growth in both gram negative and positive [22]. In another study, electrospun nanofibers based on gum Tragacanth/Poly (ϵ -caprolactone) has been produced and the nanofibers loaded with curcumin. Antibacterial tests indicated that nanofibers had a good antibacterial effect against MRSA and ESBL strains [23]. It seems that more study about Bassorin based nanofibers and its properties is needed.

The increase of specificity is the typical index of the new generation of drugs due to directing to a certain tissue, controlling of releasing speed and protection of the active agent. Over three decades polymer composites were offered as drug transporters due to various confidants like stability, superior loading capabilities and control over physicochemical properties. Another theory states that restricted drug release can be achieved by macroscopic drug near to the target site. In this regard, in situ-forming biomaterials are under attention due to the non-invasive character, decreasing of side effects related to systemic administration and control over bio-distribution [24].

Ofloxacin, 9-fluoro-2,3-dihydro-3-methyl-10-(4-1methyl-1-piperazinyl)-7-oxo-7H-pyrido [1,2,3-de]-1,4 benzoxazine-6-carboxylic acid [25] is a second generation fluorinated quinolone, a pyridine carboxylic acid derivative which exert a broad-spectrum having antimicrobial effect in a diversity of systemic putrefactions [26-28]. It blocks bacterial DNA synthesis by inhibiting DNA gyrase and topoisomerase II. Inhibition of DNA gyrase prevents the relaxation of positively super coiled DNA that is required for normal transcription and replication [29].

In the present study, producing of a new bio-fabrics was investigated by Bassorin via electrospinning method.

Firstly, the produced nanofibers has been examined by XRD, FTIR and SEM microscopy. Secondly, the antibacterial activity of nanofibers were tested by Ofloxacin compound.

EXPERIMENTAL

Materials

In the present study, Iranian Gum Tragacanth (*Astragalus Gossypinus*) was collected from plants growing in Iran. The raw gum was ground and sieved. Powdered gum with a mesh size between 200

and 500 μ m was used in this study. Poly Ethylene Oxide (PEO) with average molecular weight (Mw) of 600,000, powder Ofloxacin (Ofx) were obtained from Sigma-Aldrich Company and sterile Cotton gauzes with standard number (ISIRI 3061) product of MT Co. The efficacy of Nano wound dressing on full thickness wound model in rat was evaluated.

Solution preparation

The raw gum was grounded and sieved. Powdered gum with mesh size between 200 and 500 μ m was used in this study. Gum prepared by mixing the powder with distilled water under gentle stirring at room temperature for 2 h and then the dispersions were stored at room temperature for 24 h to allow for complete hydration of biopolymer. The crude Gum (1g) powder was wetted with 100 ml deionized water and, the mixture were stirred at room temperature up to become a smooth mixtures after 24 h.

Separating Soluble and Insoluble Fractions of Gum Tragacanth

Centrifugation at 5000 rpm for 20 min allowed to separate of soluble from swell able fraction. Gum Tragacanth consists two major fractions: a water-soluble part which contain Tragacanthic Acid and small amount of an Arabinogalactan and water insoluble part, contain Bassorin which water-swell able fraction. The Bassorin part was freeze dried, and consider for future analysis.

Electrospinning method

Bassorin/PEO blend solutions were then prepared by mixing the two solutions at 50/50 wt% .Ba/PEO nanofibers were prepared by electrospinning technique. Nanofibers solution were 0.75% Bassorin and 6.5% PEO were mixed in 1:1 Ba/PEO mass ratio and the MIC were 0.015 mg/l for Ofloxacin. Deionized distilled water was used as PEO solvent. The mixture was electro spun with 15 kV practical voltage, 20 cm needle to collector distance, and 1.5 ml/h discharge rate. At the next step, a sterile Cotton gauze with standard number (ISIRI 3061) was coated by a layer of Ba/PEO/Ofx.

XRD Diffraction Analysis

For collecting XRD data, X-ray diffract meter (X'Pert Pro MPD) was used. The pattern was recorded using Cu K $_{\alpha 1}$ ($\lambda = 0.154056$ nm) radiation at a tube voltage of 40 kV and an amperage of 30

mA. The scanning was executed in a region of 2 from 0° to 80° at 0.02°/min [30]. XRD assay was done for GT powder, freeze dried Bassorin and Ba/Ofx solution.

FTIR Analysis

Fourier Transform Infra-Red (FTIR) spectroscopic of the electro spun nanofibers were used by a Spectrum RX I spectrometer (PerkinElmer, USA) [31]. This test used for PEO/Ba nanofibers and PEO/Ba/Ofx.

SEM Microscopy

Morphology of produced nanofibers of (Ba/PEO/Ofx) and Cotton gauze coated of (Ba/PEO/Ofx) nanofibers were investigated by SEM microscopy (SEM, XL30-SFEG, FEI Philips). For this assay, the sample was coated with gold (JEOL JFC-1200 fine coater, Japan) at an accelerating voltage of 20000 V [31].

Qualitative Antibacterial Activity Analysis (AATCC147)

The antibacterial activities of cotton gauze coated electro spun nanofibers samples, were examined against *Staphylococcus aureus* (ATCC 66538), and *Escherichia coli* (ATCC 25922) via the Parallel Streak Method (AATCC 147–1998). The bacterial suspensions prepared with Try tone Soya Broth (CM0989, Oxide) at the dilution 0.5 McFarland (1.5×10^8 CFU/ml). After Mueller-Hinton agar plates preparation (105437, Merck/sterilization at 120°C for 20 min), each strain was cultured in five parallel lines using a 4-mm inoculating loop. The PEO/Ba/Ofx samples (2.5×5 cm² in size) were lightly pressed over the five inoculum streaks in the transverse. The dishes were incubated at 37°C for 24 hours and the disruption of growth sideways the streaks were studied [32].

Quantitative Antibacterial Activity Analysis (AATCC100)

Refer to the AATCC 100-2004 standard method, spherical samples of electro spun fabric, 4.8 ± 0.1 cm in diameter, were laid into a 250 ml wide-mouth glass jar with a screw cap. The samples were inoculated by 1.0 ml of a nutrient broth culture having $1-2 \times 10^5$ CFU of bacteria. An untreated sterile gauze sample was used as a control. The samples were incubated at 37 °C for 24 h. Then, the bacteria were washed from the samples by shaking them in 100 ml of neutralizing dilution for 1 min. Serial dilutions of bacterial suspension were made with sterilized water, and the suspensions

were mixed with nutrient agar in petri dishes. Subsequent, the plates were incubated at 37 °C for 24 h. Afterward, the number of colonies in each plate was calculated, and the reduction rate of bacteria, R, were calculated from:

$$R = (B - A) / B \times 100$$

R: Reduction rate.

A: The colonies in plate after 24 h.

B: The colonies in plate immediately after inoculation (at “0” contact time)[33].

Also, the antibacterial activity of the Ofloxacin treated nanofibers has been examined quantitatively by AATCC100-2004 method.

Clinical Trial on Rat

Wound healing method: To create burns, after anesthetizing the rats, injections of ketamine and xylazine into the peritoneum of the hair in the back area were shaved. The area was then disinfected with alcohol, and a 2-degree surface burn of 2 cm 2 surface area was cut by a 2-second contact and without a pressure of a piece of aluminum on the site, which was created in 96 ° boiling water. Burning room was the animal support room. The temperature and time used in this study were the result of the information obtained from previous work. After burning with a temperature of 96 °C and a 4 second contact, one of the mice was sampled and a second-degree second -degree burns was confirmed. In order to prevent shock, 3 ml of normal saline was injected into the peritoneum and after microscopic examination, the pathologists were examined by microscopic examination after the animals recovered and returned to the cages and 24 hours later, and according to clinical signs , Type II burns were confirmed in all specimens. The animals were dressed every 24 hours and the animals were taken care of the animals during the day from the morning until complete re-attachment was performed.

RESULTS AND DISCUSSION

The morphology of electro spun nanofibers

Bassorin solution does not have any ability to spin such as fibers which shown in Fig. 2 A and Fig. 2 B shows the nanofibers of Poly Ethylene Oxide with uniform fibers. Fig. 2 C shown the best uniform nanofibers by composite of Bassorin/PEO by weight ratios of 50/50. So by adding of PEO on Bassorin can help to spin the mixtures of Bassorin/PEO. The solutions were forage into about 20 mL syringe fitted with a needle. The feeding rate of the

syringe pump changed from 0.25 to 2 mL/h. High voltage in the range of 15 kV was applied using a power supply. Ba/PEO electro spun nanofibers were deposited and collected on the accumulator plate with the distance of 20 cm from the needle peak.

XRD Patterns

The XRD of the samples shown in Fig. 3. This figure shows the XRD patterns of Raw Gum (A), Bassorin (B), Bassorin/PEO (C), in Bragg's Angles 2θ between (0-90). As in Fig. 3 (A) in $2\theta=19$ a semi crystal structure of Gum Tragacanth, but the semi

crystal has not seen in pattern B. The results shows that by disappearing of semi crystal in patterns B and C and will be increased amorphous phase. So it will be help to absorb more waters in wounds exocrine liquid.

FTIR Analysis

Fig. 4 shows the FTIR spectra of GT, Ba/PEO nanofibers and Ba/PEO/Ofx nanofibers. The major absorbance bands present in the spectra of GT were at 3442, 2930, 2855, 1747, 1635, 1443, 1366, 1243, 1080 and 1022 cm^{-1} . The wide band observed

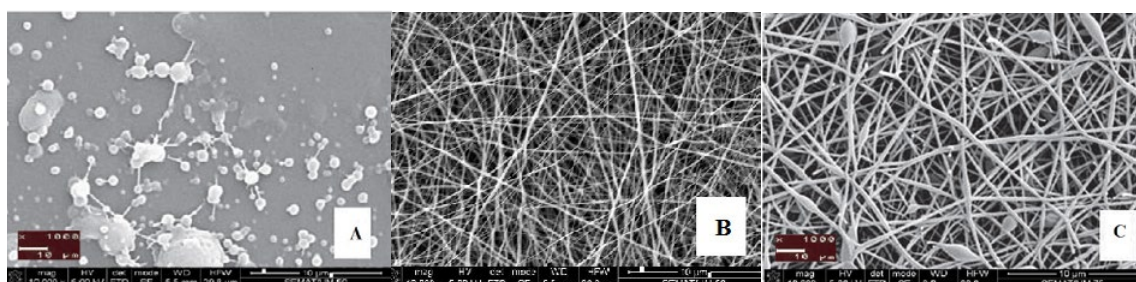


Fig. 2: SEM of Bassorin (A), Nanofibers of PEO 6.5% (B) and Nanofibers of Bassorin/PEO (C) (Magnification of all Figs 1000X)

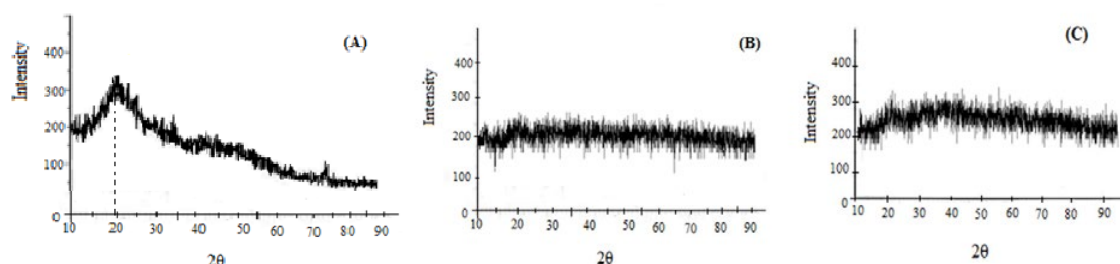


Fig. 3: XRD patterns of GT (A), Bassorin (B) and Bassorin/PEO (C)

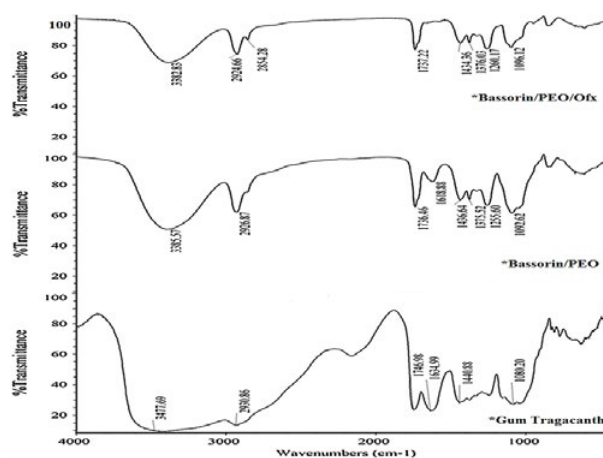


Fig. 4: FTIR spectra of Gum Tragacanth, Bassorin/PEO nanofibers and Bassorin/PEO/Ofx nanofibers

at 3442 cm^{-1} could be assigned to stretching vibrations of OH groups in the GT. The bands at 2930 cm^{-1} correspond to C-H stretching vibrations of methylene groups and the broad band at 1747 cm^{-1} shows different carbonyl species of the GT. The stronger band found at 1635 cm^{-1} could be allocated to characteristic asymmetrical stretch of carboxylate group. Bands at 1441 and 1367 cm^{-1} attributed to symmetrical stretch of C-H banding groups. The spectra of Bassorin/PEO/Ofx indicated an intense band due to the presence of hydroxyl groups (OH) at 3382 cm^{-1} . The bands corresponding to the (CH₂) asymmetric and the symmetric stretching could be showed at 2926 cm^{-1} and 2854 cm^{-1} . The bands at 2610 and 2930 cm^{-1} can be attributed to carboxylic acid stretching vibrations. The FTIR spectra of Bassorin/PEO blend nanofibers seen the characteristic peaks of GT and Bassorin/PEO/Ofx such as 1637 cm^{-1} which is attributed to asymmetrical stretch of carboxyl group. It is also observed that the bands for hydroxyl stretching become much broader with adding bassorin. This possible be due to existence of hydrogen bonding between OH groups.

Antibacterial Results

The antibacterial activity of Ofloxacin loaded nanofibers was evaluated by parallel streak (AATCC-147) method using *E. coli* (*Escherichia*

coli) and *S. aureus* (*Staphylococcus aureus*) bacteria and sterile gauze used as negative control. As it shown in Fig. 5.1. Bacterial growth under and around the negative control was witnessed, and the line of bacterial growth to the edges of the fabric can be seen. While there is no perfect zone of inhibition in line one and two of bacterial cultures but the growth of bacteria was limited under the Ofloxacin treated samples. But in case of line three to five, a very clear zone of inhibition is observed. The excellent antibacterial activity of nanofibers is due to Ofloxacin releasing into the environment. The results shown that sterile gauze coated with Bassorin/PEO can be a suitable for drug release in the wound dressing.

Qualitative test results the antibacterial activity of the produced band (AATCC 147 standard)

Quantitative results (colony count and inhibition percentage) Antibacterial activity of the produced band (standard AATCC 100)

For this test, the samples were cut into circles with a diameter of 4.8. Samples were placed in a glass container of 250 ml volume. The number of circular samples per container should be such that if 1 ml of the bacterial solution is inoculated, the suspension does not leak into the container on the other and the total inoculum solution is sampled (sample number for each different genus for example, this number is 4 for cotton fabrics). The test was used to control the

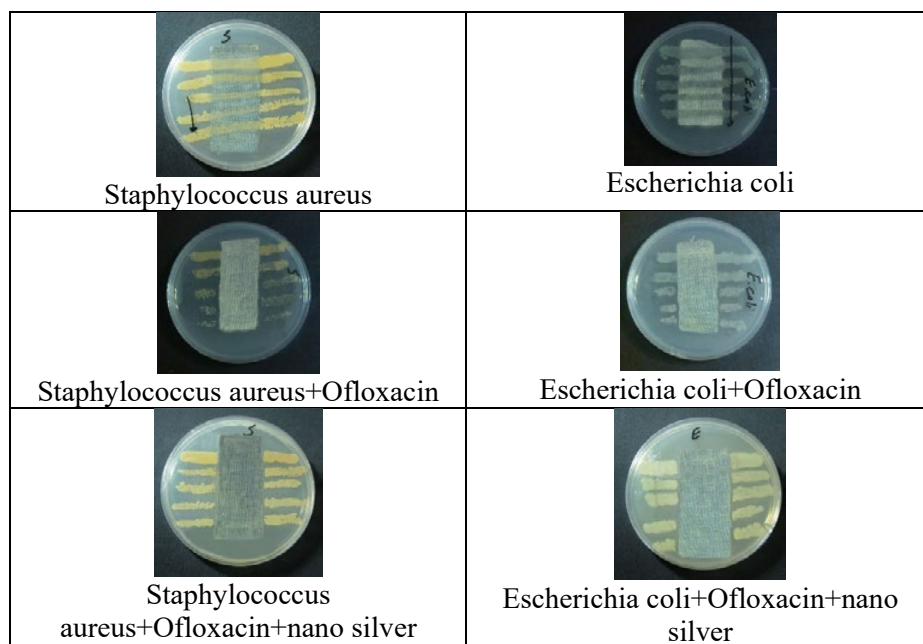


Fig. 5.1: Antibacterial assay of sterile gauze coated with Bassorin/PEO on *S. aureus* and *E. coli* [, negative control-*S. aureus*, treated sample-*S. aureus*, negative control- *E. coli* and treated sample- *E. coli*]

raw cotton raw material. Sterilization of specimens was performed by UV rays for 20 minutes for each sample. *Escherichia coli* and *Staphylococcus aureus* were used to test this test. For this purpose, 1 ml of bacterial culture was inseeded on the specimens in a glass container. The specimens were then incubated at 37 °C for 24 hours. Then, 100 ml of broth neutralizing medium was poured into each sample container and the vessel was shaken vigorously for 1 minute. The solution was then prepared in serial dilutions of 0.1, 0.01, 0.001, 0.0001 and 0.00001. In order to count the bacteria in dilutions prepared by Pour Plate method. For this purpose, each dilution of 0.1 ml was placed in a bacterial culture plate and a molten argon melt agar was added on it. In order to spread the homogeneous bacteria in the plate environment, the number was 8 English and stagnated until closure. Plates were placed in an

incubator for 24 hours at 37 °C, and then colliers were counted in each plate.

The results of Fig. 5.2 in Table 1 have been reported with three replications for *Escherichia coli* and *Staphylococcus aureus*. The number of colonies in the raw sample was average for *Escherichia coli* 196, for example, dressing with ofloxacin, 11, and nano-fiber dressing with Nano silver, 43 and finally, the dressing of nano-fibers with ofloxacin and Nano silver, 1 was reported as a sign The effect of synergistic effect on bacterial inhibition is 98.94% on dressing. Also, colony count against *Staphylococcus aureus* was reported as the average number of colonies in the raw sample for *Staphylococcus aureus* 117 for dressing with ofloxacin, 15 and the bandage sample with nano-fibers with nano silver, 41 and Finally, the band of nano-fibers with ofloxacin and nano silver was reported 3, which showed a synergistic effect

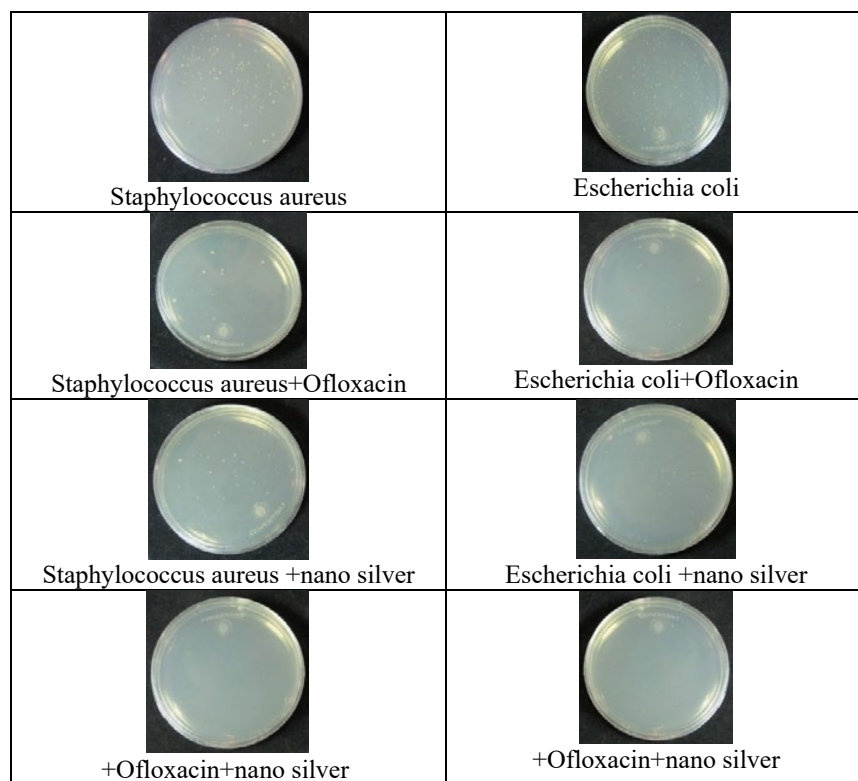


Fig. 5.2: Quantitative test results, Antibacterial assay of sterile gauze coated with Bassorin/PEO on *S. aureus* and *E. Coli*

Table 1: Antimicrobial activity of nanofibers-Ofloxacin

Sample	E. coli		S. aureus	
	Colonies	Reduction rate	Colonies	Reduction rate
Nanofibers-Ofloxacin	11	94.4%	15	87.2%
Sterile gauze	186	-	119	-

with bacterial inhibition of 97.44% on dressing.

As it observed in Table 1, the sterile gauze coated with nanofibers with Ofloxacin show high level of bacterial reduction as compared to control samples. It seems that the sterile coated gauze can release Ofloxacin in the environment and inhibits the bacterial growth.

Clinical tests on rats

To burn, after anesthesia, 230 grams of rats were injected and anesthetized with an injection of ketamine and at a dose of 50 mg / kg and 20 mg / kg . How to calculate the effective dose of the drug: $V = (\text{Kilograms} \times \text{concentration of the drug}) / (\text{animal weight} \times \text{effective dose of the drug})$
Ketamine 115 mL = $(1000 \times 100) / (230 \times 50)$ V230
xylazine 0/46 mL = $(1000 \times 100) / (260 \times 20)$ V230

Anesthesia was performed with the method on the rats and injected into the peritoneum in order to prevent the shock of 6 ml normal saline, and after coughing and returning the animals to the cages and passing 20 hours of burns by The pathologist was examined macroscopically and according to the clinical symptoms, type II burns were confirmed in all rats and then, as in the form of dressing Fig. (6).

The dressing process in 12 days with nano-fibers containing ofloxacin and nano silver in Table (2) and the dressing process in 12 days with a layer of non-drug nanofibers was reported in Table (3) by measuring the wound by the ruler. Treatment with dressing Nano-fiber containing drugs have almost doubled the recovery rate.

For better comparison, the treatment process is plotted in Fig. 7 for a period of 12 days.



Fig.6: (A) Creating superficial burns with UV rays, (B) Rat dressed, (C) Burn recovery with burn dressing

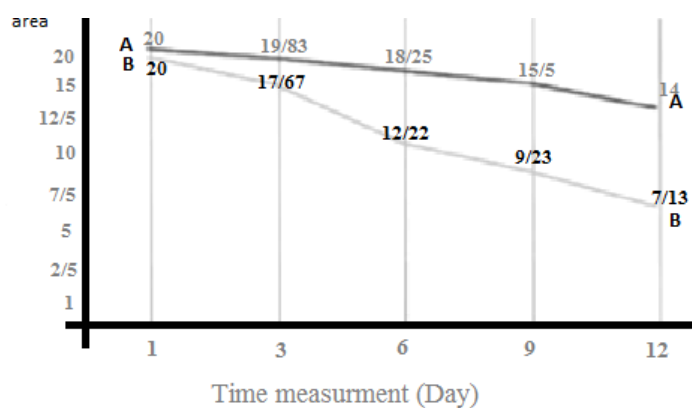


Fig. 7: Comparison of the effect of wound dressing in the control group, dressing of nano-fibers without drug (A) and dressing of nano-fibers with drug (B)

Table 2: Improvement of rats with nano-fiber dressing with drug

20 mm ²	The first day
17/67 mm ²	The third day
12/22mm ²	The Sixth day
9.23 mm ²	The ninth day
7.13 mm ²	The 12th day

Table 3: Improvement of rats with nanofibers dressing without drug

20 mm ²	The first day
19/83mm ²	The third day
18/25 mm ²	The Sixth day
15.5 mm ²	The ninth day
14 mm ²	The 12th day

CONCLUSION

In the present study, we extracted of Bassorin from Gum Tragacanth by Centrifugal process, Bassorin which water-swellaable fraction. The solution of Bassorin viscosity were very high and could not be electrospin. To make better the spin ability of Bassorin, Poly Ethylene Oxide (PEO) was mixed with this polysaccharide. The results showed that, it is possible to produce smooth surface nanofibers. The ratio of Bassorin/PEO diverse and nanofibers with best morphology were obtained by combination of 50/50 Bassorin/PEO ratios. Concentration solutions were 0.75% Bassorin and 6.5% PEO, and the condition of electrospinning mixture were electrospun with 15 kV practical voltage, 20 cm needle to collector distance, and 1.5 ml/h discharge rate. SEM spectrum shown the nanofibers of Bassorin/PEO formed after electrospinning. The XRD of Gum Tragacanth, Bassorin and Bassorin/PEO shown a semi crystal structure of Gum Tragacanth, but the semi crystal has not seen in Bassorin and Bassorin/PEO/Ofx. So it will be help to absorb more waters in wounds exocrine liquid. The FTIR spectra of Bassorin/PEO blend nanofibers showed the characteristic peaks of Bassorin and PEO. The sample showed good antimicrobial property with the Ofloxacin as an effective antibiotic, loaded in Bassorin/PEO solutions and electrospinning were done. The results showed Bassorin/PEO can forms smooth and bead free nanofibers and also as a layer placed on sterile cotton gauzes. The qualitative antibacterial assay indicated that produced nanofibers could release Ofloxacin, and inhibit the growth of bacteria under and around the location of the nanofibers. So Bassorin/PEO treated nanofibers showed acceptable antibacterial activity (about 90%) on both *E. coli* and *S. aureus* bacteria in the quantity antibacterial test. The results of the pathobiology in one week showed the highest improvement in sterile gauze band containing nano-bassorin, ofloxacin and nano silver.

Clinical tests reported a rapid recovery process, and the results in rat at 12 days showed the highest improvement in sterile cotton gauze containing nano-bassorin, ofloxacin and nano silver. According to the said articles about the absorption of water and antibacterial and drug release, the dressing produced can be used as a dressing for grade 2 burns.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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