

Extraction water-swellaible fraction of gum tragacanth for innovation in burn wound dressing

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Gum Tragacanth (GT) obtained from *Astragalus gossypinus* is one of the most widely used natural gums which has found applications in many areas because of its attractive features such as biodegradability, nontoxic nature, natural availability, moisture absorption and creating a network of hydrocolloid. It also has maintenance and delivery of drugs, higher resistance to microbial attacks and long shelf-life properties. 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 insoluble in water, has the capacity to swell and form a gel.

In present study, the separation of tragacanthin and bassorin suggests that the two polysaccharides are in a physical mixture and not chemically bonded. Bassorin which represents 60–70% of the total gum with a molar mass of approximately 105 Da, though insoluble in water. Another small fraction, termed tragacanthin is soluble in water with a molar mass of approximately 104 Da to give a colloidal, hydrosol solution. Bassorin, a pectic component, has a chain of (1-4)-linked α -D-galacturonic acid units some of which are substituted at O-3 with β -D-xylopyranosyl units and some of these being terminated with D-Gal or L-Fuc .The solution of bassorin viscosity were very high and could not be electrospun. To improve the spin ability of bassorin, Polyethylene oxide (PEO) was blended with this polysaccharide. Preparation nanofibers of 50 wt% bassorin (extracted from gum tragacanth) has been mixed by 50 wt% polyethylene 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 investigated. Nanofibers are capable of absorbing wound's exocrine liquid easily due to their high specific area of nanofibers which 4 to 5% more than cotton gauzes without nanofibers. When it is turned to gel by moisture sorption, the release of loaded ofloxacin would be enhanced. The Antibacterial assay showed the cotton gauze coated with Ba/PEO/Ofx nanofibers could inhibit about 90% growth both bacterial strain.

Key words: Hydrocolloid, Gum Traghacanth, Bassorin, Poly Ethylene Oxide, Electrospinning, Nanofibers, Antibacterial

INTRODUCTION

Since old ages, diverse herbal resources have continually had special significance and aids in the wound-healing process[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 species of *Astragalus gossypinus* which contains two main segments: water-soluble fractions namely tragacanthin (low quantity of an arabinogalactan) and water-swellaible 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, natural available and also safe resulted in the fabrication of scaffolds from GT were used for many aspects of skin healing, drug carrier, and periodontal defect regeneration[3]. GT consists 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-Dxylopyranose Figure 1A [4] and the main structural of GT, cf. Figure 1B. Basssrin structure extracted from GT shown in Figure 1C [4] .

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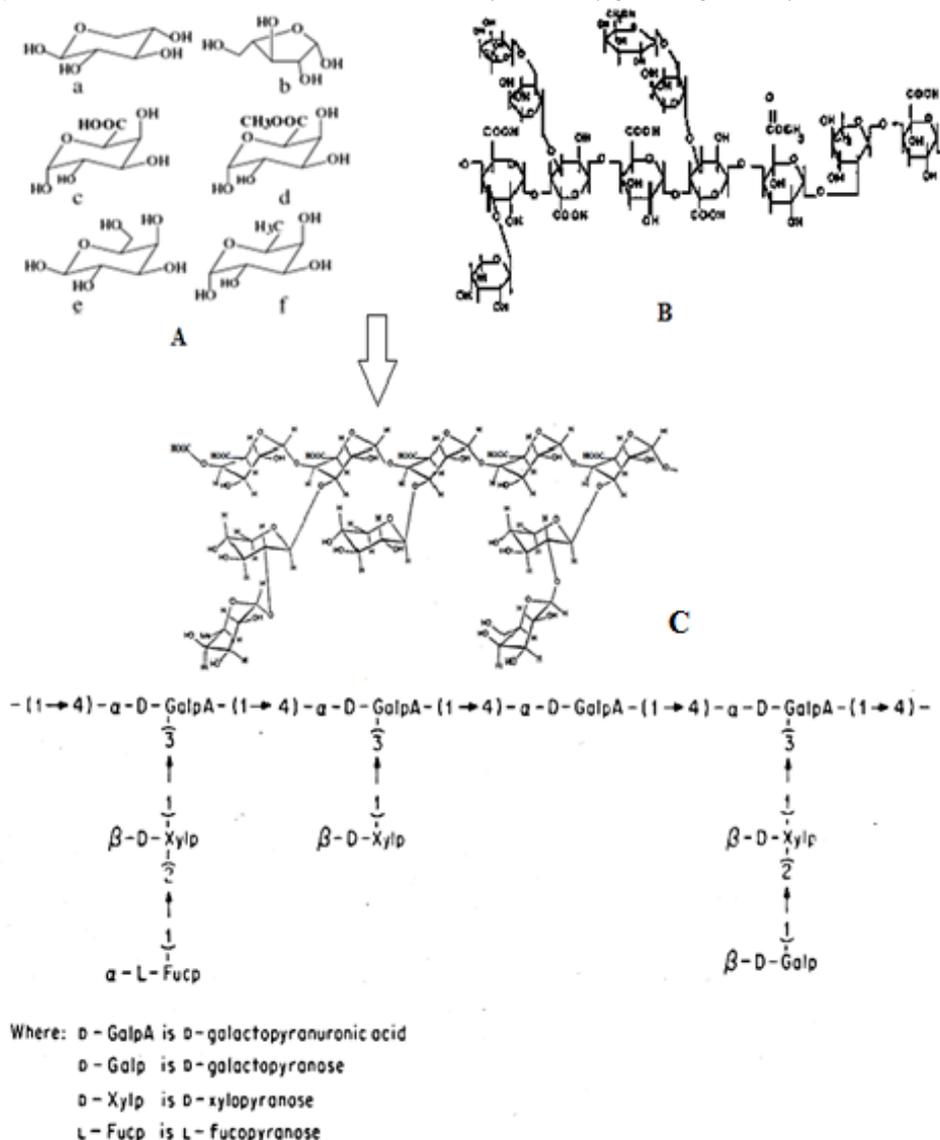


Fig. 1. (A) Main chemical building units of the Gum Tragacanth. a) β -D-xylose, b) L-arabinose, c) α -D-galacturonic acid, d) α -D-galacturonic acid methylester, e) β -Dgalactose, f) α -L-fucose, (B) The main structural of GT, (C) Structure of Bassorin [4].

It has been reported that gums tragacanth from different species of astragalus have different ratios of the two fractions, different chemical compositions and also varying physicochemical properties; therefore, different functionalities and applications for each species are expected [5]. Gum tragacanth, a highly acid-resistant hydrocolloid, has been accepted since 1961 as GRAS at the level of 0.2e1.3% [6] and has been used for many years as a stabiliser, thickener, emulsifier and suspending agent in the food, pharmaceutical, cosmetic, textile and leather industries as well as in technical applications based on its high viscosity at low concentration, good action, unusually high stability to heat and acidity and effective emulsifying properties. It also is pourable and has a creamy mouth feel and good flavour-release properties [7] and very long shelf life [8]. Rather, at least the water

soluble fraction (tragacanthin) appears to resemble pectin, and seems to contain linear chains of galacturonic acid (probably 1,4-linked); hence, gum tragacanth species rich in xylose with minor levels of fucose may contain xylogalacturonans and some fucoxylogalacturonans as the main components of the soluble fraction, whereas those having high fucose levels may mainly contain fucoxylogalacturonan in the tragacanthin part [9]. The water soluble tragacanthin is reported as a neutral, highly branched arabinogalactan comprising (1-6)- and (1-3)- linked core chain containing galactose and arabinose (both in furanose and pyranose forms) and side groups of (1-2)-, (1-3)- and (1-5)-linked arabinose units occurring as monosaccharide or oligosaccharides (Table 1) [10].

Depending on the species, the ratio of the water-swallowable to the water-soluble fraction varies [9].

Table 1. Linkage analysis of gum tragacanth (*Astragalus gossypinus*)

Monosaccharide	Linkage type	% peak area
L-Fucose	t-Fucp	23.9(69.3)
	1,3-Fucp	2.8(8.1)
	1,4-Fucp	3.2(9.3)
	1,2-Fucp	3.8(11)
	1,3,4-Fucp	0.8(2.3)
L-Rhamnose	t-Rhap	0.1(3.3)
	1,2,4 Rhap	2.7(90)
	1,4 Rhap	0.2(6.7)
L-Arabinose	t-Araf	5.2(53.6)
	1,5 Araf	3.4(35)
	1,2,5 Araf	1.1(11.4)
D-Galactose	t-Galp	1.7(29.8)
	1,4-Galp	2.6(45.6)
	1,6 Galp	0.2(3.5)
	1,4,6 Galp	0.1(1.8)
	1,3,6 Galp	1.1 (19.3)
D-Glucose	t-Glup	0.2(11.8)
	1,4-Glup	1.4(82.3)
	1,2,4-Glup	0.1(5.9)
D-Xylose	t-Xylp	7.2(21.2)
	1,2,4-Xylp	0.8(2.4)
	1,2-Xylp	24.9(73.5)
	1,2,3-Xylp	1(2.9)
D-Galacturonic acid	t-GalAp	0.1(3.3)
	1,4-GalAp	2.8(93.3)
	1,4,6-GalAp	0.1(3.3)

Gum tragacanth solutions are acidic, usually in the pH range of 5-6. Its maximum initial viscosity is at pH 8, but usually exhibited maximum stability near pH 5 [8].

The gum tragacanth is considered as generally recognized as safe (GRAS) at the 0.2–1.3% level in food stuffs in the USA since 1961. It is also approved as a food additive in European Union and has the number E413 in the list of additives confirmed by the Scientific Committee for Food of the European Community [11]. This biodegradable and biocompatible biopolymer is not allergenic, mutagenic, teratogenic and carcinogenic with no adverse toxicological effects in non-allergic people [11-13]. Several researchers have reported the application of GT in different fields such as the green synthesis of silver nanoparticles [14], immobilizing

agent in viral plaque assay [15], hydrogel membranes [16], dressing for healing of burn wounds [17], super absorbent hydrogel [18] and carrier for controlled release of drugs, etc. [19]. Khajavi and coworkers produced GT fibers by solution spinning method via alkaline treatment and they investigated the effects of spinning parameters on the mechanical properties of produced fibers [20,21].

A straightforward and efficient method of obtaining ultra-fine fibers is electrospinning technique with diameters ranging 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 [22-24]. It has been displayed chitosan biopolymer able to wound healing in human and also shown significant antibacterial activity against various type of bacteria [24-25].

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 [26]. In recent decades, antibiotics are known as an intense source of medicinal agents for infections treatment [27]. In a similar study, Ranjbar-Mohammadi et al. investigated PLGA/Gum 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 [28]. 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 [29]. 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 [29, 30].

Ofloxacin,9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperaziny)-7-oxo-7H-pyrido[1,2,3-de]-1,4 benzoxazine-6-carboxylic acid [31] is a second generation fluorinated quinolone, a pyridone carboxylic acid derivative which exert a broad-spectrum having antimicrobial effect in a variety of systemic infections [32-34]. It blocks bacterial DNA synthesis by inhibiting DNA gyrase and topoisomerase IV. Inhibition of DNA gyrase prevents the relaxation of positively super coiled DNA that is required for normal transcription and replication [35].

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. Polyethylene 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.

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 separated of soluble from swellable 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-swellable fraction. The bassorin part was freeze dried, and consider for future analysis shown in Figure 2.



Fig. 2. Gum tragacanth fractions

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 electrospun 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_{α1} ($\lambda = 0.154056$ nm) radiation at a tube voltage of 40 kV and an amperage of 30 mA. The scanning was executed in a region of 2θ from 0° to 80° at 0.02°/min [36]. XRD assay was done for GT powder, freeze dried Bassorin and Ba/Ofx solution.

FTIR analysis

Fourier Transform Infrared (FTIR) spectroscopic of the electrospun nanofibers were used by a Spectrum RX I spectrometer (PerkinElmer, USA) [37]. 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 [37].

Qualitative antibacterial activity analysis (AATCC147)

The antibacterial activities of cotton gauze coated electrospun 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 Tryptone Soya Broth (CM0989, Oxoid) 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 [38].

Quantitative antibacterial activity analysis (AATCC100)

Refer to the AATCC 100-2004 standard method, spherical samples of electrospun 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) [39].

RESULTS AND DISCUSSION

The morphology of electrospun nanofibers

Bassorin solution does not have any ability to spin such fibers shown in Figure 3A. Figure 3B shows the nanofibers of polyethylene oxide with uniform fibers. Figure 3C shown the best uniform nanofibers by composite of bassorin/PEO by weight ratios of 50/50. Hence, addition of PEO to bassorin can help to spin the mixtures of bassorin/PEO. The solutions were feed into a 20 mL plastic syringe fitted with a needle. The feeding rate of the syringe pump changed from 0.5 to 1.5 mL/h. High voltage in the range of 15 kV was applied using a power supply. Ba/PEO electrospun nanofiber were deposited and collected on the collector plate with the distance of 20 cm from the needle tip.

XRD Patterns

The XRD of the samples shown in Figure 4. 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 figure 4 (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-crystals in patterns B and C and the amorphous phase will be increased. So it will help to absorb more waters in wounds exocrine liquid.

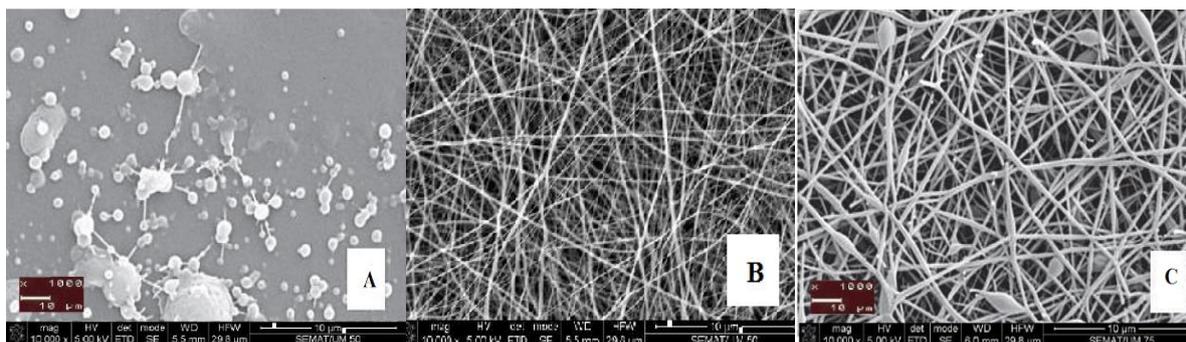


Fig. 3. SEM of bassorin (A) , nanofibers of PEO 6.5% (B) and nanofibers of bassorin/PEO (C) (magnification of all figures 1000 x).

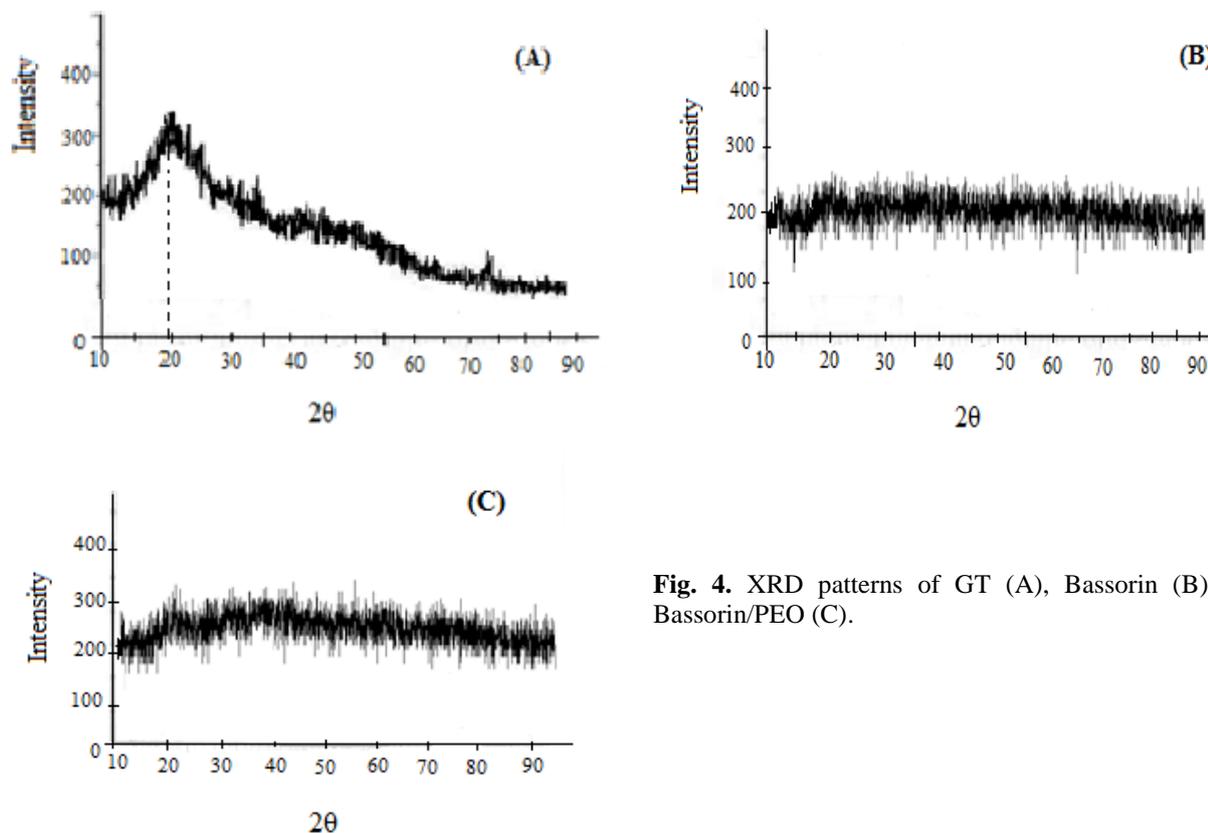


Fig. 4. XRD patterns of GT (A), Bassorin (B) and Bassorin/PEO (C).

FTIR Analysis

Figure 5 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, 2856, 1747, 1635, 1441, 1367, 1243, 1080 and 1023 cm^{-1} . The broad band observed at 3442 cm^{-1} could be assigned to stretching vibrations of OH groups in the Gum Tragacanth. The bands at 2930 cm^{-1} correspond to C-H stretching vibrations of methylene groups and the broad band at 1747 cm^{-1} shows various carbonyl species of the gum. The stronger band found at 1635 cm^{-1} could be assigned 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 seen at 2925 cm^{-1} and 2854 cm^{-1} . The band at 2610 and 2930 cm^{-1} can be attributed to carboxylic acid stretching vibrations. The FTIR spectra of bassorin/PEO blend nanofibers showed 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 may be due to presence of hydrogen bonding between OH groups.

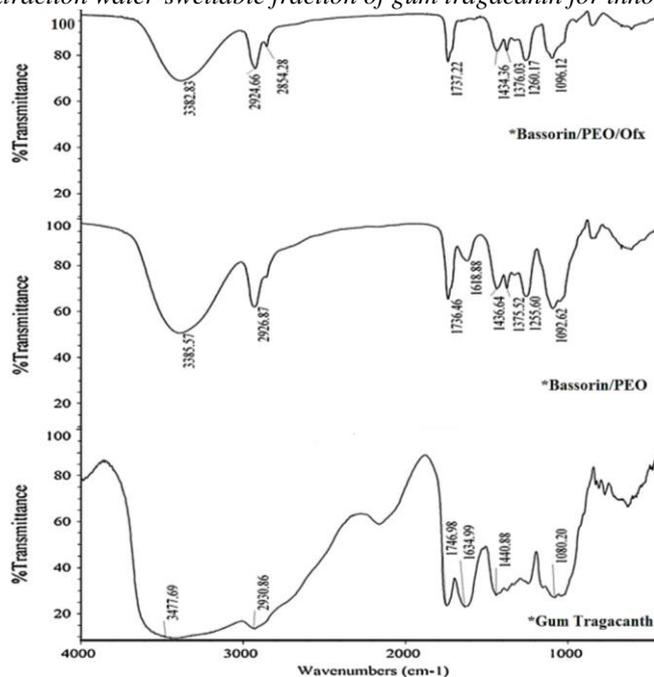


Fig. 5. FTIR spectra of Gum Tragacanth, Bassorin/PEO nanofibers and Bassorin/PEO/Ofx nanofibers

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 Figure 6. 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.

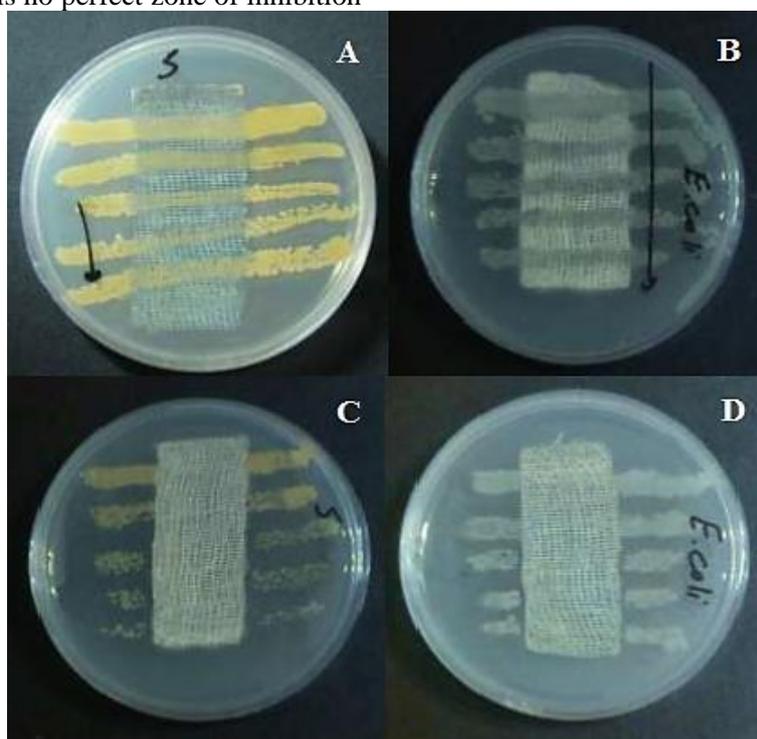


Fig. 6. Antibacterial assay of sterile gauze coated with bassorin/PEO on *S. aureus* and *E. coli* [(A) negative control-*S. aureus*, (B) treated sample-*S. aureus*, (C) negative control-*E. coli* and (D) treated sample-*E. coli*]

Also, the antibacterial activity of the ofloxacin treated nanofibers has been examined quantitatively by AATCC100-2004 method. As it observed in Table 2, the sterile gauze coated with nanofibers

with ofloxacin shows 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.

Table 2. Antimicrobial activity of nanofibers-ofloxacin

Sample	<i>E. coli</i>		<i>S. aureus</i>	
	Colonies	Reduction rate	Colonies	Reduction rate
Nanofibers-ofloxacin	11	94.4 %	15	87.2 %
Sterile gauze	186	-	119	-

CONCLUSION

In the present study, we extracted bassorin from gum tragacanth by centrifugal process, Bassorin contains water-swellaable fraction. The solution of Bassorin viscosity were very high and could not be electrospun. To improve the spin ability of bassorin, polyethylene oxide (PEO) was blended with this polysaccharide. The results showed that, it is possible to produce smooth surface nanofibers. The ratio of bassorin/PEO varied 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.

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