Preparation and characterization of Poly vinyl Alcohol blend solutions with natural extract oils and walnut bark aqueous solution for antibacterial application

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Abstract

In this study, it was examined what would happen if one volume of a mixture of polyvinyl alcohol (PVA), starch (STA), and cress seed gum (CSM) was added together with an aqueous solution of walnut bark at various weight percentages (0.3, 0.5, and 0.7 percent wt). (30STA, 30CSM, 40PVA) via electrospinning. Tests on the viscosity, electrical conductivity, and surface tension of the solution were conducted to determine its impact on the fiber diameter. The pumping parameters were set to the following values: (20 kV applied voltage, 20 cm pumping distance, 0.4 mm needle diameter, 600 rpm target rotation speed, and 1 ml/hour pumping rate). A scanning electron microscope was used to look at the tissue and determine the structure and shape of the produced nanofibers. Tests on the tissue included the FTIR infrared test to determine the bonding between the mixture and the addition. Results showed that adding aqueous solutions of walnut bark with concentrations of 3 percent and 5 percent resulted in a decrease in the diameter of the fibers and the smoothness of their surface, while adding a concentration of 7 percent resulted in an increase in the diameter of the fiber and a rise in the contact angle. According to the findings of the bacterial inhibition assay, it was demonstrated that the concentration of the aqueous solution containing the powdered walnut bark increased the ability to inhibit bacteria

Keywords: walnut bark , blend solutions, antibacterial , PVA
DOI Number:10.14704/nq.2022.20.8.NQ44461 **NeuroQuantology 2022; 20(8): 4263-4276**

Introduction

The understanding of bioactive polymers is becoming increasingly crucial today. Numerous studies on the physicochemical makeup and functional characteristics of various bioactive polymers have been published. These studies make it possible for the industry to choose the appropriate biopolymers without having to make a lot of mistakes [1]. As a result, studies into the creation and characterization of packaging made of biodegradable and petroleum-derived polymers, which is a crucial component in the food industry, have significantly increased, primarily as a result of interests in reducing the environmental impact that using synthetic packaging materials has [2-3].

The availability of natural and synthetic polymers including cellulose, chitosan, lignin, starch, polylactic acid (PLA), polyvinyl alcohol (PVA), alginic acid and derivatives, etc. has led scientists to place a special emphasis on biopolymers. Consequently, these materials need to be nontoxic, renewable, and have the required characteristics. However, the use of the electrospinning process to produce films from these polymers has grown recently [3]. Micro- to nanoscale fibers can be created using the electrospinning technology. Due to its cost-effectiveness, adaptability to a wide range of materials, ability to control fiber morphology, and ease of scaling, electro spinning is unmatched as a method [4-8].

The Nanocomposites based polymer matrix is comprised of two phases, the first of which is a polymer matrix that can be a laminate or fiber, and the second of which is a reinforcing material that is in the nanoscale range and includes nanoparticles, pigment, plant dyes, and DNA. [9-14]

Synthetic and natural biopolymer blends are essential for developing innovative materials with a range of applications and for enhancing properties like low cast and high operation efficiency [15]. On the other hand, by adding natural nano particles, can achieve fresh enhancement of outcome blend properties. [16-17]

Like a result of the excellent biocompatibility and physicochemical properties of this biopolymer, researchers have recently focused a lot of emphasis on the development of starch-containing nanofiber [18]. Starch is a common and extensively used biocompatible polymer that is utilized for food packaging due to its excellent biodegradability and low cost. Their inadequate mechanical and barrier properties in conditions like high relative humidity, however, limit their applicability. Consequently, it is thought that blending starch with other biopolymers will aid in improving these properties of starch [19].

Cress seed (Lepidiumsativum) belongs to the Brassicaceae family. Cress seeds rapidly absorb water to form a viscous mucilage. These seeds contain around 6.5%–15% mucilaginous materials [14]. This mucilage is composed of hydrocolloids and enhance mechanical strength, tensile strength, flexibility, and water vapor barrier properties, mucilages have been used for the encapsulation of bioactive compounds in food packaging applications. Cress seed mucilage is rich in two sugars: mannose (38.9%) and arabinose (19.4%) [20-21].

Starch (STA) and cress seed mucilage (CSM) cannot be spun alone and needs to be used with an electrospinning aid agent. Polyvinyl alcohol (PVA) is a semi-crystalline hydrophilic inexpensive, biocompatible and non-toxic polymer with excellent chemical and thermal stability. Polyvinyl alcohol (PVA) was used as an aiding agent for fiber spinning due to the presence of a hydroxyl group in its structure [22-23].

Recently, there is a growing interest in applying the electrospinning technique for the production of nanofibers that contains active packagin ingredients, such as plant compounds, due to their enhanced antibacterial, anti-inflammatory, and antioxidant properties [24]. These plant compounds as walnut tree stem bark. Walnut tree (Juglansregia L.) stem bark belongs to juglandaceae and contains chemical constituents, namely β-sitosterol, ascorbic acid,
juglone, folic acid, gallic acid, regiolone, and quercetin-3-α-L-arabinoside [25].

The dried stem bark possesses anti-inflammatory, blood purifying, and anticancer properties. It has contain several therapeutically active constituents, especially polyphenols. the dried walnut bark has significant amounts of total phenol content compared to its prepared extracts (34.83–311 and 9.8 mg/g) respectively [26].

Antibacterial and antioxidant activities can be found in nano metals, their oxides, and their ions. These elements function naturally to stop oxidation and limit bacterial development and are included in many natural products.[27]

Nano-metals, their ions, and their oxides have been used in numerous prior studies as bacteria-inhibiting materials and in a variety of applications, such as packaging and filtering.

In the present study, the electrospinning method was utilized to fabricate nanofiber from the mixture of (STA), (CSM) and (PVA) as aid agent with addition walnut bark (WB) as powder to investigate the physicochemical, antibacterial and antioxidant properties of the fabricated nanofiber.

Experimental part:

Materials used:

The samples were made using poly vinyl alcohol (PVA), which has a molecular weight range of 13000–23000 g/mol and an 88 percent hydrolysis rate, according to central drug house (P) Ltd. From an Iraqi market, we bought Alpha Chemika, product corn starch (STA), cress seed, and walnut tree bark. The solvent used to create the electrospinning solutions was distilled water.

Cress seed mucilage (CSM) extraction:

Mucilage was extracted by cleaning the seed to remove any contaminants. The cress seed (5 to 100 (w/w)) was then added to deionized water, which was mixed with a magnetic stirrer and kept at 100º for 15 minutes. After that, a cloth filter was used to separate the seed from the mucilage in the solution. The mucilage solution is then allowed to cool before being utilized in the experiment. The constituents detected in this mucilage were analyzed in the Babylon agriculture directorate/central laboratory,as shown **in the table (1)**

Table 1: illustrates the characteristic of the cress seed mucilage

Ca (ppm)	Mg (ppm)	K (ppm)	Na (ppm)	pH
250	25	236.5	4.1	6.1

Walnut tree bark powder (WB)

The bark of the walnut tree (Juglandacea) was acquired from an Iraqi market and washed before being dried for one hour at 40 degrees Celsius to remove any impurities. After that, it was grinded with a stainless steel grinder to make it simple to dissolve in solutions, which promises to release Ca ions through these solutions and alter their characteristics (viscosity, electrical conductivity, and surfaces tension). It was then dried in an oven at 40° C for an hour. The powder was then sieved using sieves with a granular size of 90 m and analyzed by EDX to determine the elements included in it, as shown in figure 1a. The powder was further examined by AFM and a particle size analyzer to determine the powder's grain size, as shown in figures 1 b and c.

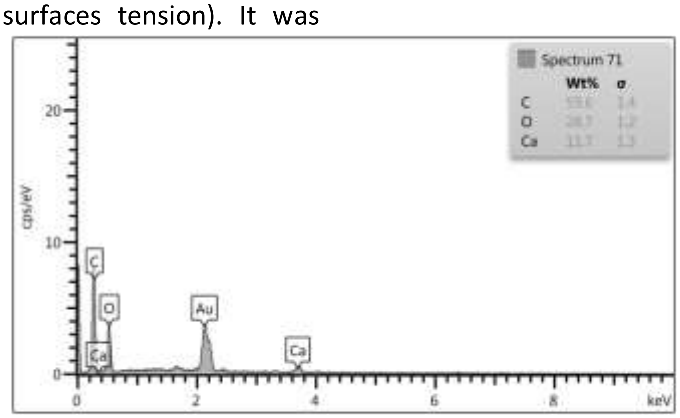


Fig. (1a.): EDX of the walnut bark powder.

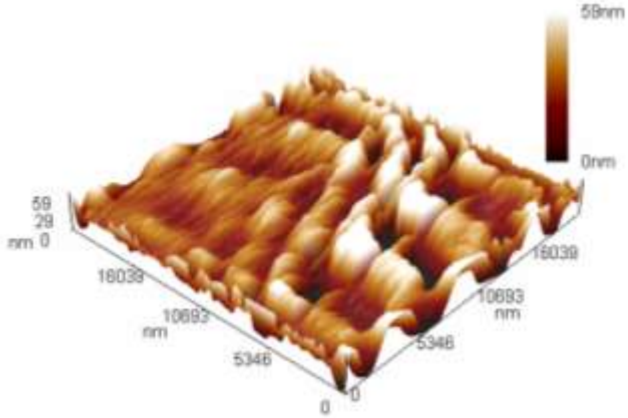


Figure 1.b AFM image of walnut bark powder

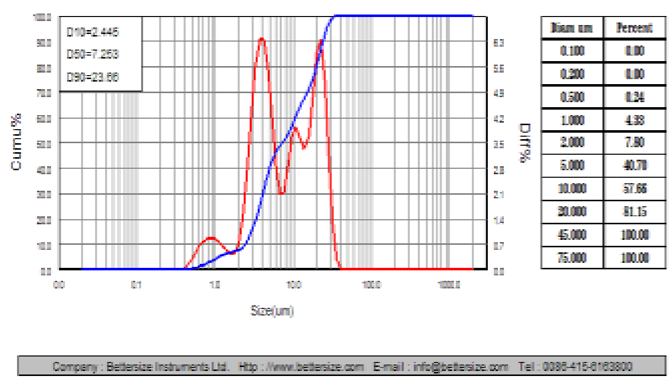


Figure 1.c Particle size distribution image of walnut bark powder

Figures 2b and 2c show that the walnut powder's particle size ranges from 500 nanometers to 15 micrometers.

Fabrication of samples for electrospinning

The first step starch gelatinization solution was prepared by dissolving (2.5g) starch in (100 ml) of hot distilled water at 90 ^{o}C for 15 minutes (0.025 con. w/v). Cress seed mucilage solution with a concentration of 5 g. PVA solution prepare by dissolve (8g) in (100 ml) of hot distilled water at 80^{o}C for 90 minutes under magnetic stirrer to obtain a concentration of (0.08w/v). Afterward, (STA+CSM+PVA) were mixed together at volume ratio (30:30:40) for one hours at room temperature using magnetic stirrer to obtain homogenies solutions. The second step walnut tree bark (WB) powder was added in different percentage (0.3, 0.5, 0.7 wt.%) and blended with (30:30:40) for 3hr. at room temperature. The concentrations were determined using the mixing rule. Table 2 shows the samples prepared for electrospinning method.

Table (2): show the samples and solution concentration ratios under electrospinning settings.

Sample No.	Contents ratios %	Walnut tree bark powder (WB) wt. %	Con. w/v	Electrospinning conditions
S1	PVA:STA:CSM (40:30:30)	0	0.054	HVPS = 20 kV E.D =20 cm

S2	PVA:STA:CSM (40:30:30)	0.3	0.3g/100ml	N.D = 0.4 mm Temperature = 20ºC R.S = 600 rpm F.R = 1 ml/hr RH% (25-40)
S3	PVA:STA:CSM (40:30:30)	0.5	0.5g/100ml	
S4	PVA:STA:CSM (40:30:30)	0.7	0.7g/100ml	
HVPS: applied voltage, E.D: electrospinning distance, N.D. needle diameter , R.S : rotate speed of collector , F.R : Flow rate				

The solution changed color from yellow to a dark nutty color when walnut powder was added to the previously produced solution in the amounts stated in Table 2. This was caused by the pigment in the powder, which was dissolved in the solution. Fig 2.

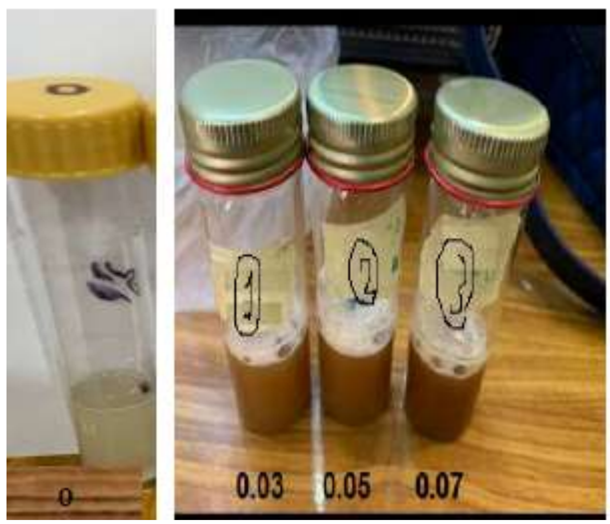


Figure 2. electro spun solutions

Results and Discussion

Solution results:

As shown in figure (3-a) and table 3, the viscosity decrease after adding WB in different percentage . This is because the viscosity of the aqueous solution of the walnut powder is low, and more calcium ions are generated, which function as viscosity reducers by assisting in the sliding of the liquid's layers and lowering shear resistance. On the other hand, a greater WB ratio causes the viscosity of the solution to increase because the solution's intensity of contents increases, preventing the movement of polymer chains and increasing the shear resistance of the solution.

Table (3): The physical properties of samples

S. No.	Con%	Viscosity (cP)	Electrical conductivity (µS/cm)	Surface tension (mN/m)

S1	0.054	390	695	38.6
S2		384	771	44.06
S3		365	776	50.25
S4		371	780	52.52

As demonstrated in Fig 3-b, adding (WB) aqueous solution to this blend (40PVA:30STA: 30 CSM) increase the electrical conductivity with increasing the WB percent because the ionic nature of WB powder, Which agrees with the results published by [22-23].

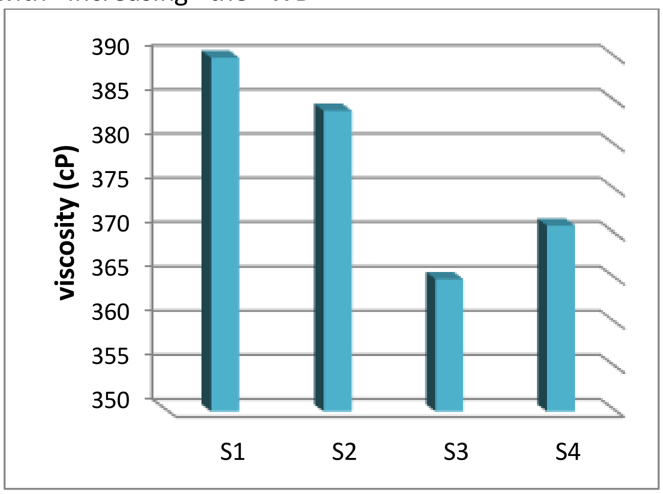


Fig. (3-a): show viscosity of the samples

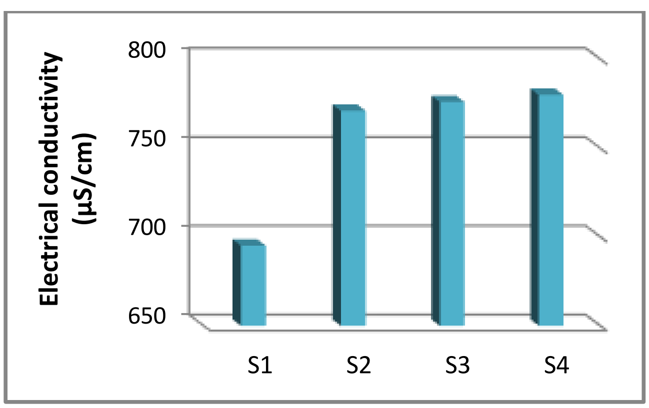


Fig. (3-b): show electrical conductivity of the samples

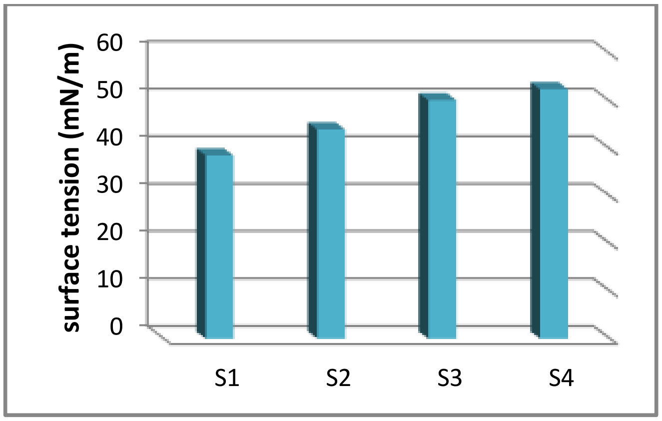


Fig. (3-c): show surface tension of the samples

Fig (3. a-c): illustrates the physical properties of samples/ (a) viscosity,(b)/ electrical conductivity and (c)/ surface tension.

FTIR results:

FTIR analysis was used to observe functional groups of the interaction between S1 and WB powder. The FTIR spectra of WB pure, S1 and S3 are illustrated in figure 6. In this figure notice some peaks for WB pure which presented by the broad peak in 3425 cm^{-1}corresponding to the stretching vibrations of O–H in hydroxyl groups of phenols and the stretching of N–H in amine groups.

The peak 2931 cm^{-1} and 2368 cm^{-1} refer to the stretching of C–H in alkane and that of C≡C groups, respectively. Then, the peak at 1620 cm^{-1}, 1442 cm^{-1} , 1319 cm^{-1} , 1064 cm^{-1} , 887 cm^{-1} and 779 cm^{-1} is attributed to the (carbonyl) stretching vibrations of C=O in polyphenols, C-H bend, C-N stretching vibration, C-O stretching vibration in amino acids, C-C stretch and C-H aromatic groups, respectively. therefore the WB rich in polyphenols and amino acids and this consistent with the research of Ning Wang et al. [24]. Meanwhile the FTIR spectra of S1 show the peak at 3425 cm^{-1} , 2368 cm^{-1} , 1625 cm^{-1}, 1435 cm^{-1} , 1103 cm^{-1} and 894 cm^{-1} is correspond to the O–H stretch, C-H stretch, C=O (carbonyl) stretching vibrations, C-H bend, C-O stretching vibration and C-C stretch respectively. Thus the FTIR spectra of (S1+WB) which represent (S3) has the same spectra of S1 that indicates to good embedding ability and stable structure. But notice the peak at 3425 cm^{-1} shifts towards the larger wavenumbers and this shifts lead to intermolecular interactions between S1 and WB which represent by hydrogen bonds between the carbonyl groups of S1 and the hydroxyl groups of WB which agrees with Ning Wang et al. [18]. As well as the carbonyl index which refer to the thermo oxidative degradation for sample that was noticed from the intensity of carbonyl band at (1625 cm-1). This the intensity decrease after adding WB due to its nature antioxidant which reduced from thermo oxidative degradation of sample as agreement research Rouba and others [29].

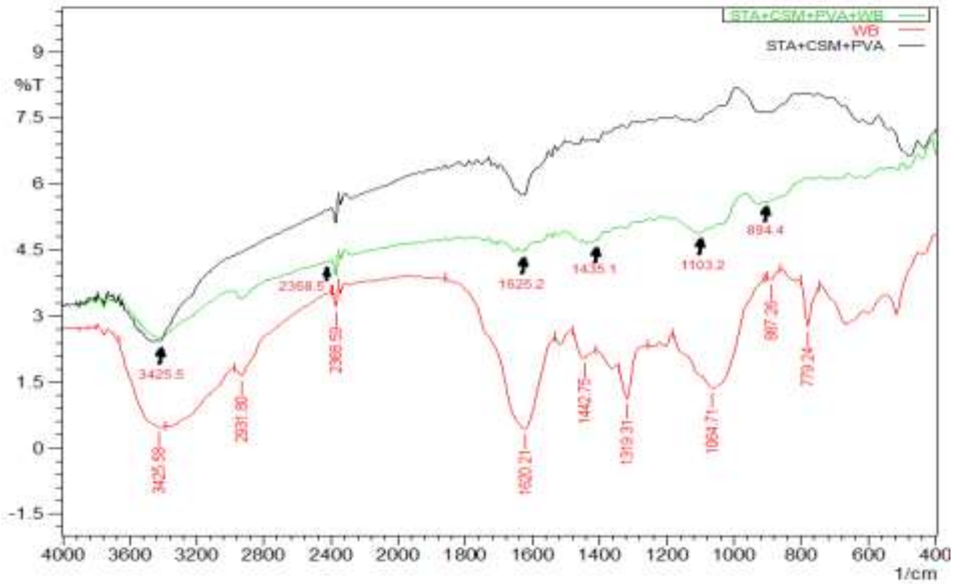


Fig. (7): FTIR spectra of S1,WB pure and S3.

Contact angle:

Samples of nanofibers' contact angles are shown in Figure 4 and Table 4.

Table 4 shows contact angles of the samples.

Sample No.	Contact angle (ºC)
S1	74
S2	76
S3	78
S4	65

Because tree barks contain significant amounts of lignin, which plays a structural role in plant cell walls and also provides hydrophobicity characteristics [30], there is an increase in contact angle after adding WB with ratios (0.3 and 0.5 percent). However, this increase is still within the range of hydrophilicity due to the OH bonds that were presented through mixing of solution samples. In accordance with Tonello et al. 2021 [31], the ratio (0.7 percent) then caused a reduction in contact angle, which resulted in a greater concentration of OH bonds in the sample.

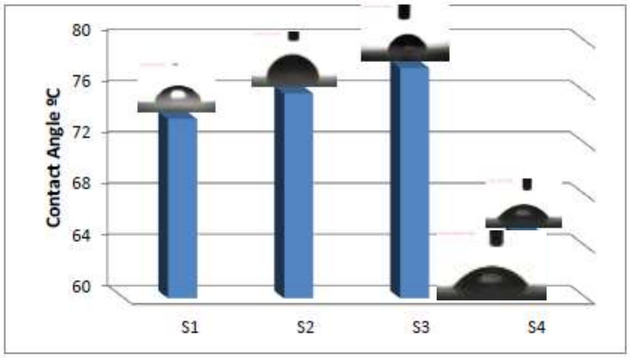
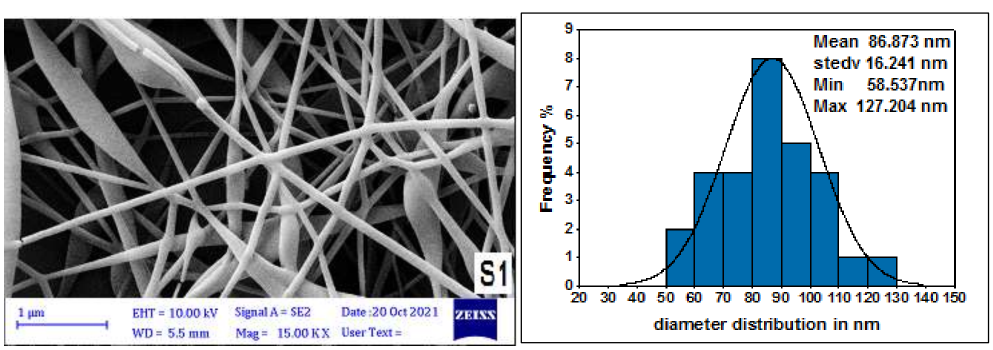


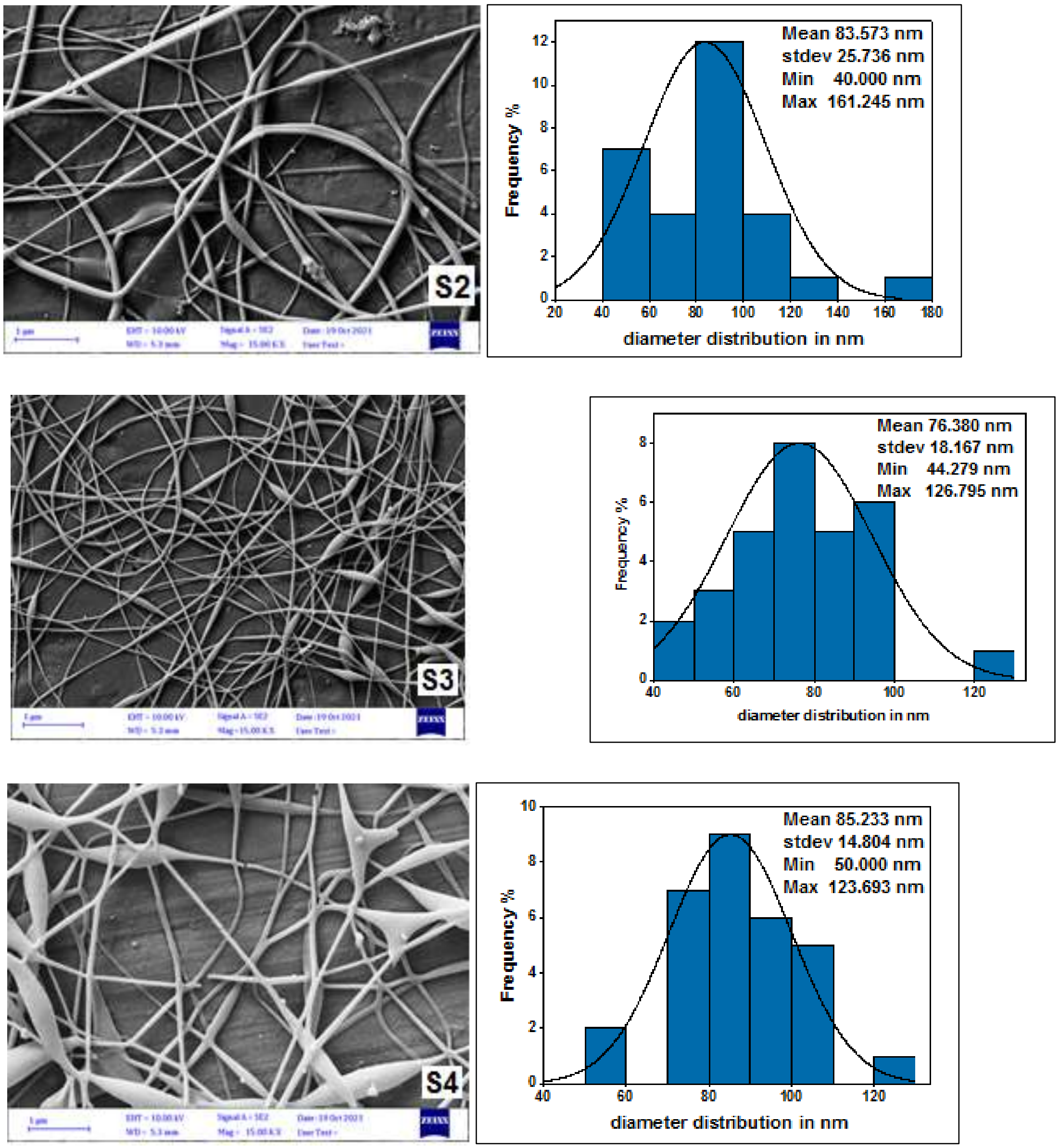
Fig. (4): show contact angle of the samples.

FESEM results:

Figure 5 displays the nanofiber samples' FESEM pictures. The electrical conductivity of the electrospinning precursor solution increases with an increase in the amount of WB, leading to an increase in the strength of the electric field during the spinning process, which causes the diameters of the nanofibers to decrease after adding WB in S2 and S3. , fibers with lower diameters are preferred. While the diameters of nanofibers grow after adding (0.7 percent WB) in S4 because of high viscosity, this is consistent with Mwiiri et al. 2020 [31-32] in that high viscosity induced resistance to jet stretching with an unstable Taylor cone and discontinuous jet during the electrospinning process.

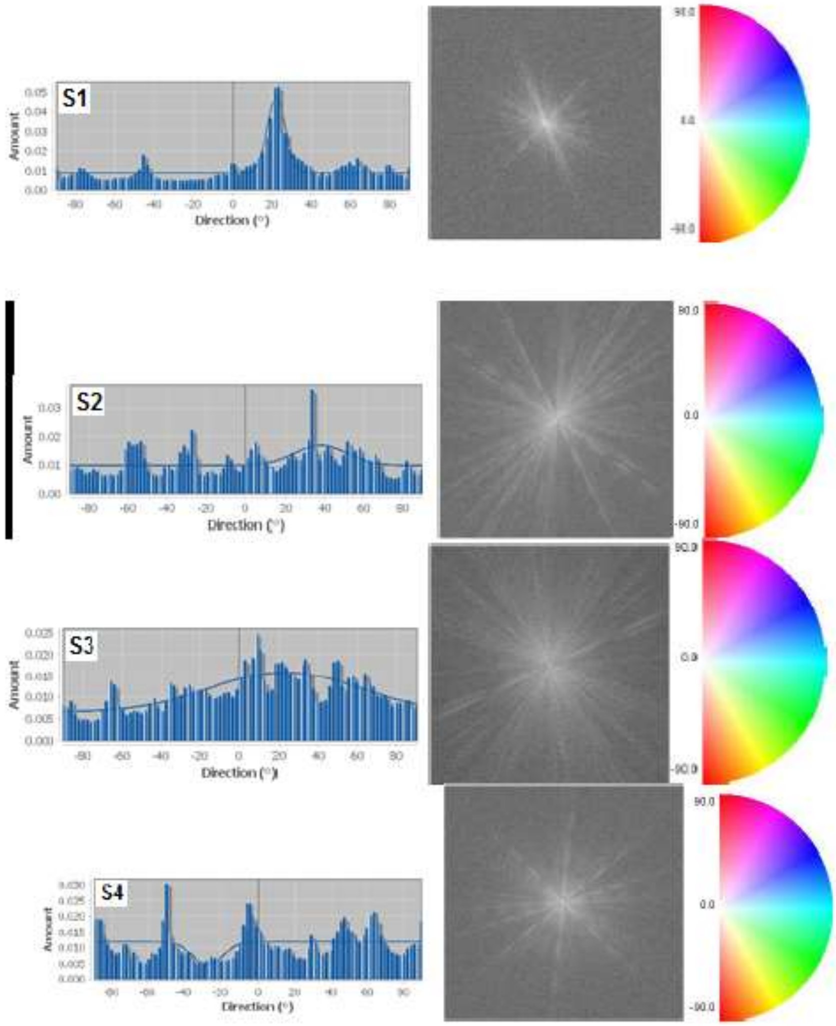
Figure (6) and table (5) show directionality histograms and directionality analysis of the samples, which show the directed fibers amount in the angle (22.17º) for S1 was 28% while in the S2 and S3 increase to 37% and 67% at angle (37.75º, 23.04º) respectively and this indicate to increase in crystallinity of samples as well as an increase in contact angle. But show decrease in the amounts of directed fiber for S4 which indicate to decrease in crystallinity and contact angle of this sample as shown in figure (4) .





FE-SEM micrographsNanofibers diameter distribution histograms

Figure (5):FESEM Micrographs the magnification power of 100 nm and Nano-fibers diameter distribution histograms for S1, S2, S3 and S4.



Directionality histograms 2D-FFT

Figure (6): Directionality histogram, and 2D-FFT for (A) S1(PVA), (B) S4 (PVA+STA),(C) S7 (PVA+CSM) and (D) S8(PVA+STA+CSM).

Table (5): The directionality analysis for the samples (by utilize Fourier components)

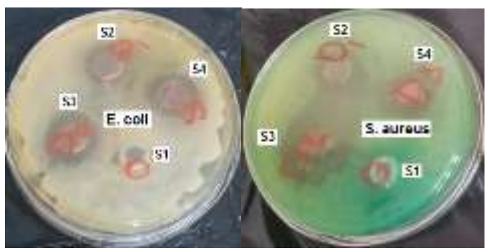
Sample No.	Direction (˚)	Dispersion (°)	Amount	Goodness
S1	22.17	4.54	0.28	0.74
S2	37.75	13.47	0.37	0.48
S3	23.04	37.49	0.67	0.56
S4	-27.00	8.55	0.20	0.33

Antibacterial activity results:

Antibacterial activity against Staphylococcus aureus and Escherichia coli, representing gram-positiveand -negative bacteria, respectively tested by Mueller Hinton (MH) agar was prepared and poured into Petri dishes and allowed to cool down and a loop full of each microorganism was inoculated on the surface of a separate MH agar plate. The antimicrobial activity was determined by the agar-well diffusion method. Figure 10 and table 8 illustrated the antibacterial activity and diameter of inhabitation of samples which increased after adding WB as an antibacterial agent due to the nature of WB and this agreement with Ning Wang et al. 2022 [32].

Table (8): Antibacterial results of the samples.

Sample No.	E. coli Zone Diameter (mm)	S. aureus Zone Diameter (mm)
S1	0	0
S2	17	13
S3	20	20
S4	20	15



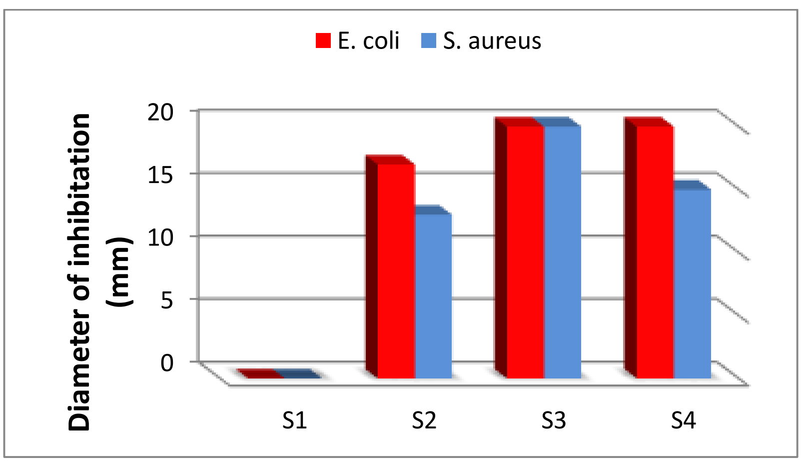


Fig. (11): show diameter of Inhabitation for the samples

Conclusions:

Results proved there are an decrease in the diameter of the fibers and the smoothness of their surface after adding the aqueous solution of walunt bark with 3 % and 5 % concentration , while the addition of 7 % concentration led to an increase in the diameter of the fiber, in addition to an increase in the contact angle. As for the results of the bacterial inhibition assay, it was proved that the ability to inhibit bacteria increased when the concentration of the aqueous solution of walnut bark powder was increased.

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