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Antimicrobial characteristics of wool fibers treated with chitosan-propolis nano composite and dyed with natural dye extracted from Red Prickly Pear

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Abstract Nanotechnology provides a new concept for the improvement of the antimicrobial activity of the textile fibers. Natural dye extracted from red prickly pear using microwave heating was used for dyeing wool fibers. Chitosan-Propolis nano composite was applied as treatment on wool fibers before dyeing by using microwave and ultra sonication methods. The dye concentration and pH factors as well as color strength, color data and fastness properties of the dyed wool fibers were investigated. The results indicated that wool fibers pretreated with the tested nanomaterials exhibited better results than the untreated samples. Nano composite of chitosan and propolis showed antimicrobial activity against some pathogenic fungi and bacteria. The results indicated that the antimicrobial activity of the natural dye under investigation was good and it was enhanced by treatment with the nano composite. The morphological structure of the untreated and pretreated wool fibers was examined by scanning electron microscopy (SEM). The untreated wool fibers have a rough surface. The pretreated wool fibers were swollen as compared to the untreated fibers. The diameter of the fibers increased, and the surfaces became smooth and even.

Keywords: Nano chitosan, Propolis, Red prickly pear, Natural dye**,** Antimicrobial activity

Introduction

Bacteria and fungi, either pathogenic or not, are generally found on human skin, nasal cavities, and other parts of the body. Microorganism may spread into a textile material either directly in clothes or on surrounding textiles. It is one of the most probably causes of hospital infections. Recent studies indicated that contamination of textiles may lead to the spread of pathogens to the air, then it infests the surrounding environment. Pathogenic microorganisms as *Pseudomonas aeuroginosa*, *Staphylococcus epidermidis, Staphylococcus aureus* and *Candida albicans* have been_found on textiles (Ali *et al*., 2011).

In addition, microorganism can cause malodors, stains and damage of mechanical properties of the fibers that could cause the product to be less

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effective in textile industry. It may promote skin contamination, inflammation and in sensitive people, may cause atopic dermatitis. The use of antimicrobial textiles may significantly reduce the risk of infections especially when they are used in close contact with the patients or in the surroundings. Antimicrobial agents are natural or synthetic compounds that inhibit the growth of microorganism because they are protein, lipid or enzyme inhibitors, all of which are essential for cell survival or kill the microorganisms through damage of the cell wall. Almost all antimicrobial synthetic agents on textiles are biocide. Chitosan which is deacetylated chitin is non-toxic, resistant to microorganisms, biodegradable and biocompatible. The antimicrobial activity of chitosan is affected by some factors such as the type of chitosan, the degree of deacetylation, molecular weight and other factors such as pH, ionic strength and non-aqueous solvents. Chitosan can be considered an antimicrobial agent for textile finishing (Shin *et al.*, 2010; Dutta, *et al.*, 2002). The application of chitosan in textile industry is effective against a wide range of microorganisms only at high concentrations. In recent years, antimicrobial textiles have gained interest from both academic research and industry because of their ability to produce high-quality life and safety benefits to people. Textile products are prone to microorganisms that cause diseases, unpleasant odors, color degradation and deterioration of textiles. Antimicrobial textiles can be used to produce many products such as sportswear, outdoor apparels, undergarments, shoes, furnishings, hospital linens, wound care wraps, towels and wipes. Several test methods have been developed to determine the efficacy of antimicrobial textiles such as dynamic shake test (quantitative methods like serial dilution and plate count method) and agar diffusion test (qualitative method such as halo method) (Shin *et al.*, 2010).

Propolis (bee glue) is a sticky resinous substance produced by honey bees from different plant sources such as leaves, flowers, and bud exudates, modified by bee secretions and wax (Simone-Finstrom *et al*., 2017). Several groups of researchers documented that all types of propolis expressed antibacterial properties which has antibacterial effect against both Gram-positive and Gram-negative microorganisms including multidrug-resistant bacteria such as Methicillin-resistant *Staphylococcus aureus* (MRS). The aim of this research work was to determine the effect of pretreatments of wool fibers with chitosan-propolis nano composite on enhancement of dyeing and antimicrobial activity.

Materials and Methods

Wool fibers10/2 and fibers were supplied by El Mahalla Company, Egypt. Chitosan low molecular weight and liquid propolis were purchased from ELGmhoria company, Egypt. Ultrasonic, Thermostatic CREST benchtop 575 HT ultrasonic cleaner of capacity 5.75 L, frequency 38.5 kHz and with a maximum 500 Watt output was used. The output power levels are from 100 up 500 Watt. Microwave, the microwave equipment used in this experiment was Samsung M 245 with an output of 1,550 watts operating at 2450 MHz. This research was carried out in National Research Center, Textile Research Division, Dyeing and Printing Department, Cairo, Egypt in 2019.

Preparation of chitosan-propolis nano particles

Chitosan nanoparticles encapsulated with liquid propolis were prepared by ionic gelation method with modification (Koukaras *et al.*, 2012). Different concentrations of chitosan solution (0.5–1% w/v) were prepared in 0.1% v/v glacial acetic acid and filtered. Sodium tri polyphosphate solution (TPP) (0.2% w/v) was prepared in distilled water. Liquid propolis (0.5–1.0 mg/mL) was added to chitosan solution (0.5–1% w/v). containing 0.5% w/v Tween 80 and then added together under constant stirring to obtain different formulations of chitosan-propolis nanoparticles. The mixture was then sonicated for 5 minutes and the TPP solution was added drop-wise under constant stirring. The ratio of chitosan:TPP solution was maintained at 2:1 throughout the experiment. The supernatant obtained was subjected to ultracentrifugation at 25000 rpm for 20 minutes to sediment the chitosan-propolis conjugated nanoparticles, which were then subjected to further characterization.

Pretreatment of wool fibers with chitosan/ propels nano composite

Wool fibers were treated with pad-dry-cure technique. The wool fibers were treated with chitosan-propolis nano composite at the concentrations of 2-10 ppm, padded to 100% wet pick up, dried at 80 ℃ for 5 min and then cured at 120 ℃ for 3 min.

Scanning electron microscopy (SEM)

The surface morphology of untreated and treated wool fibers were investigated by using scanning electron microscopy (SEM) with a JSMT-20, JEOL-Japan. Before examination, wool fibers surface was prepared for an appropriated disk and randomly coated with a spray of gold. SEM was carried out at the National Research Centre (Egypt).

Dyeing process

Dyeing wool fibers were done using microwave method. In a dye bath containing 2% dye extracted from red prickly pear (Fig. 1) with liquor ratio 30:1, wool fibers were dyed using microwave heating at different pH values

(3-9) for different duration (1-5 min.) The dyed samples were rinsed with cold water, washed in a bath at liquor ratio 60:1 using 3 g/L non-ionic detergent (Hostapal CV, Clariant) at 50 ℃ for 30 min then rinsed and finally dried at ambient temperature.

Dyeing wool fibers using ultra sonication method: Wool fibers were dyed with 2% of dye extracted from red prickly pear using ultra sonication at L.R 1:50, pH 5. The samples were dyed at temperature of 50^0C for (30min.), the power energy of ultra sonication was 500watt, then the samples were rinsed with cold water, washed in a bath containing 5g/l non-ionic detergent, at 50 ℃ for 30 minutes, then rinsed and dried in air at room temperature.

Figure 1. Fruits of red prickly pear

Measurements of antimicrobial activity

The antimicrobial activities of wool fibers dyed with dye extracted from red prickly pear pretreated with chitosan-propolis nano composite was evaluated by using standard methods (serial dilution and plate count method (Ben-David and Davidson, 2014). Exactly 1gm of each fiber sample was added to 99ml sterile distilled water to form the stock solution (blank) at concentration 10^{-1} from which serial dilutions (10^{-1} to 10^{-5}) were prepared. To investigate and count the bacterial strains, nutrient peptone Agar (NPA) media was used. For testing of the fungal strains, potato dextrose agar (PDA) media was selected. Exactly 0.1 ml from each dilution (10^{-1}- 10^{-5}) was spread on previously sterilized petri-plates and used in triplicates according to the standard spread plate technique, for both bacterial and fungal strains. The (NPA) plates were then incubated at 37 ℃ for 24 h and the PDA plates were incubated at 27 ℃ for 72 h. After successful growth of microorganisms, characteristics of each distinct colony, e.g., shapes, color, transparency, etc. were determined. Gram stain was performed to observe the cellular morphology and gram reaction of the bacteria. The number of bacterial and fungal colonies in the fiber samples was counted and the density was expressed as colony forming units (CFU).

The antimicrobial activity was estimated and expressed as reduction in total count of fungus and bacteria in each treatment. The isolates of pathogenic bacteria and fungus used are *Staphylococcus aureus* (G+), *Escherichia coli* (G+), *Pseudomonas aeruginosa* (G-) and *Aspergillus niger.*

Results

Effect of chitosan –propolis nano composite

Wool fibers dyed with natural dye extracted from red prickly pear and pretreated with chitosan-propolis nano composite were evaluated. The results obtained indicated that, the pretreatment using 1.5 and 2 ppm concentration of composite of chitosan and propolis gave the highest values of K/S for dyed wool fibers by microwave and ultrasonication methods. The result of pretreatment with chitosan–propolis nano composite by microwave and ultrasonication methods is shown in Tables1 and 2.

Table1. Color data of dyed wool fibers and pretreated with chitosan-propolis nano composite by ultrasonication method

Conc. of the composite (ppm)	K/S	L*	a*	b*	C*	ΔH	Δ E
0.5	10.32	59.20	19.92	9.09	21.90	24.53	42.93
1.0	6.52	51.11	31.52	14.58	34.73	24.82	52.58
1.5	15.82	50.3	35.7	15.8	35.74	25.7	55.78
2.0	16.34	42.5	36.7	16.8	37.73	26.5	57.58
Untreated	7.61	53.90	35.05	20.94	40.83	30.85	30.12

Table 2. Color data of dyed wool fibers and pretreated with chitosan-propolis nano composite by microwave method

Conc. of the composite (ppm)	**K/S**	**L***	**a***	**b***	**C***	**ΔH**	**ΔE**
0.5	2.69	63.30	43.91	4.83	44.17	6.27	56.76
1	4.27	44.75	43.92	10.15	45.08	13.02	51.10
1.5	23.32	60.66	56.92	20.05	60.35	19.41	71.24
2	7.83	42.51	52.79	14.10	54.64	14.95	63.89
2.5	5.17	49.65	49.93	14.85	52.02	16.28	59.08

Color data of dyed wool fibers with the dye extracted from red prickly pear and pretreated with chitosan-propolis nano composite at different concentrations

The colorimetric data (L*, a* and b*) of different fibers dyed with different concentrations of extracted dye was done by microwave and ultrasonication methods as seen in Tables 1 and 2. It can be concluded that the increase in extracted dye concentration used, there was a decrease in L* values and thus the color of the samples got darker. As the dye concentration increase, a* and b* values increased in the positive direction. The color of dyed wool fibers turned to more reddish yellow color and became darker with increased dye concentration from 0.5 to 2 ppm.

Effect of pH on the color strength for dyed wool fibers pretreated with nano composite

The color strength of wool fibers dyed with natural dye extracted from red prickly pear was affected by pH. The dye has high affinity to the fibers in acidic medium. In acidic medium, the cationized amino groups can adsorb anionic dye molecules by the electrostatic attraction. Result showed that the color strength of wool fibers dyed with the dye under investigation gave the highest value of color strength (K/S) at pH 5 for ultrasonication method and at pH 4 for microwave method (Fig. 2).

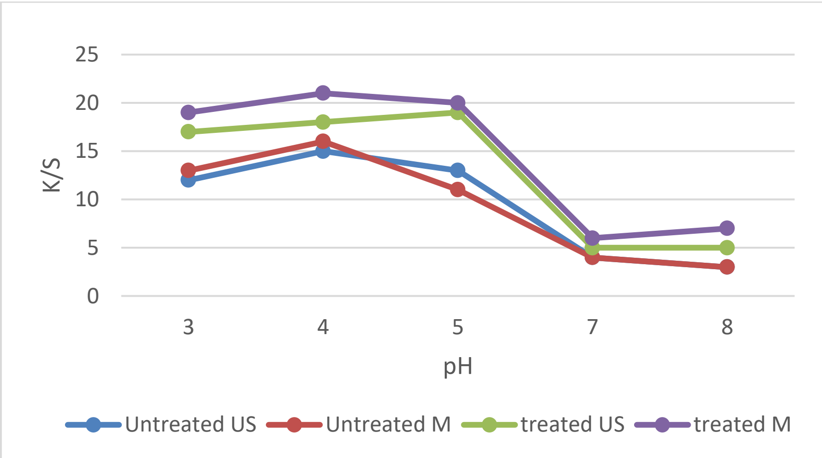

Figure 2. Effect of pH on the color strength for dyed wool fibers pretreated with the nano composite and untreated dyed fibers using microwave and ultrasonicationation

Effect of time on the color strength for treated and untreated dyed fibers by microwave method

The effect of time of dyeing wool fibers pretreated with chitosan – propolis nano composite and dyed with red prickly pear using microwave method resulted the highest values for the color strength was after 5 min (Fig.3).

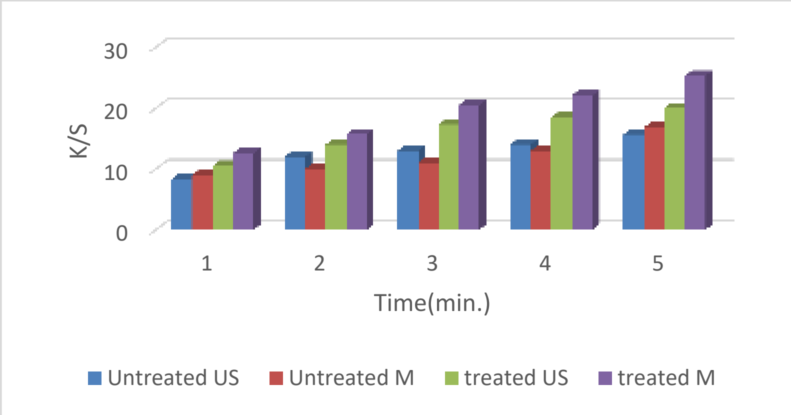

Figure 3. Effect of dyeing period on the color strength for wool fibers pretreated with nano composite and untreated dyed with red prickly pear using microwave and Ultrasonicationation

Antimicrobial Activity

The isolates of pathogenic bacteria and fungus including *Staphylococcus aureus* G+**,** *Escherichia coli* G+, *Pseudomonas aeruginosa* G- and *Aspergillus niger* was evaluated the antimicrobial activity of wool fibers dyed with natural dye from red prickly pear in combination with chitosan -propolis nano composite and natural dye. It showed that the antimicrobial activity of wool fibers depended on the concentration of chitosan and propolis (Table 3).

Table 3. Antimicrobial activity of dyed wool fibers pretreated with different concentrations of chitosan–propolis, nano composite and dyed by microwave and ultrasonication methods

microorganism	**Growth reduction (%) of different microbes on fibers treated with chitosan at concentration (ppm)**					
		0	**1**	**1.5**	**2**	2.5
S. aureus G+	Microwave	12	20	50	52	90
	Ultrasonication	12	30	50	54	85
P. aeruginosa	Microwave	15	30	35	55	80
	Ultrasonication	20	45	54	65	75
A. niger	Microwave	15	40	50	55	78
	Ultrasonication	20	57	53	65	65
E. coli G-	Microwave	7	15	25	30	76
	Ultrasonication	12	25	47	60	50

Control: untreated wool

Minimum inhibitory concertation (MIC)

The investigation of the MIC of the tested material chitosan and propolis was done to determine the optimal concentration at which the maximum inhibition was achieved. The result is represented in Figure 4 which indicated that the tested nano materials varied in their effects on the tested microorganisms. The MIC for *Streptococcous* was at 3ppm. On the other hand the MIC for the other tested strains (*E.coli*, *P.aeruginosa*, *A. niger*) was 3.5 ppm. The minimum inhibitory concentration(MIC) of chitosan-propolis nano composite was shown in Table 4 and Fig.4.

Table 4. Minimum inhibitory concentration(MIC) of chitosan-propolis nano composite

Microorganism	**Conc.**	**ppm**					
	1	1.5	2.	2.5	3	3.5	4
Staphylococcus aureus G+	12	30	50	54	90	93	95
Escherichia coli G-	12	25	47	60	67	70	73
Pseudomonas aeruginosa	20	45	54	65	75	80	84
Aspergillus niger	20	57	53	65	65	70	70

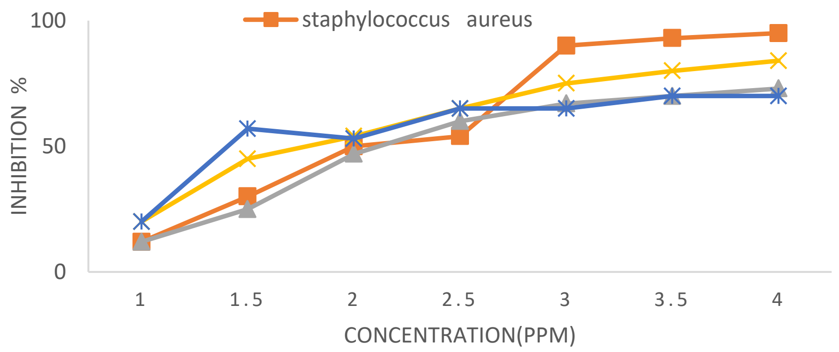

Figure 4. Minimum inhibitory concentration of the chitosan –propolis nano-composite

The fastness properties

The fastness properties of the investigated dye on the wool fibers pretreated with chitosan –propolis nano-composite at different concentrations by ultrasonicationation and microwave methods were revealed in Tables 5 and 6.

Table 5. The fastness properties of the investigated dye on the wool fibers pretreated with chitosan at different concentrations by ultrasonicationation method

Conc.g/L	Rubbing fastness		washing fastness		Perspiration Fastness				Light fastness
	Wet	Dry	Alt.	S. c	Alkaline Alt	S.c	Acidic Alt	S. c	
0	4	4-5	4	4	4	4-5	4	4	5
1	4	4	4	4	4	4	4	4-5	6
2	4-5	4-5	4-5	4-5	5	5	4-5	4-5	6
3	4	5	4-5	5	4-5	5	4-5	5	5
4	4-5	5	4-5	5	4-5	5	4-5	5	7
5	4-5	5	4-5	5	4-5	5	4	5	7
	5	4-5	4-5	4-5	4	4-5	4-5	4-5	7

Alt: Altration, Sc: Staining cotton

Table 6. The fastness properties of the investigated dye on wool fibers pretreated with chitdsan-propolis nano composite at different concentrations by microwave method

`Conc.g/L	Rubbing Fastness		Washing fastness		Perspiration Fastness				Light fastness
	Wet	**Dry**	**Alt.**	**S. c**	**Alkaline Alt**	**S.c**	**Acidic Alt**	**S. c**	
0	4	4-5	4	4	4	4-5	4	4	5
1	4	4	4	4	4	4	4	4-5	6
2	4-5	4-5	4-5	4-5	5	5	4-5	4-5	6
3	4	5	4-5	5	4-5	5	4-5	5	5
4	4-5	5	4-5	5	4-5	5	4-5	5	7
5	5	5	4-5	5	4-5	5	4-5	5	7

Alt: Altration, Sc: Stainning cotton

Surface Morphology

Surface morphology of the chosen formulation was viewed using transmission electron microscopy (TEM) (Figure 5). The nanoparticles were found to be spherical in shape with an average particle size 108.5 nm with a standard deviation of 25.46 nm. Although the average particle size measured by photon correlation spectroscopy is 247 nm, it was an estimation based on scattered light intensity. The actual physical measurement is done by TEM. The chitosan-propolis nanoparticles were found to have a smooth surface with no surface drug crystals.

The morphological structure of the untreated and pretreated wool fibers was examined using scanning electron microscope (SEM). The effect of treatment with 4g/L concentration of chitosan and propolis as seen in the SEM is shown in Figures 6 and 7 for the untreated and treated wool fibers respectively. The untreated samples have a rough surface. The treated samples were swollen compared to the untreated fibers, the diameter of the fibers increase and have smooth and even surfaces. The changes in the surface morphology was due to the effect of active ingredients chitosan–propolis nano composite.

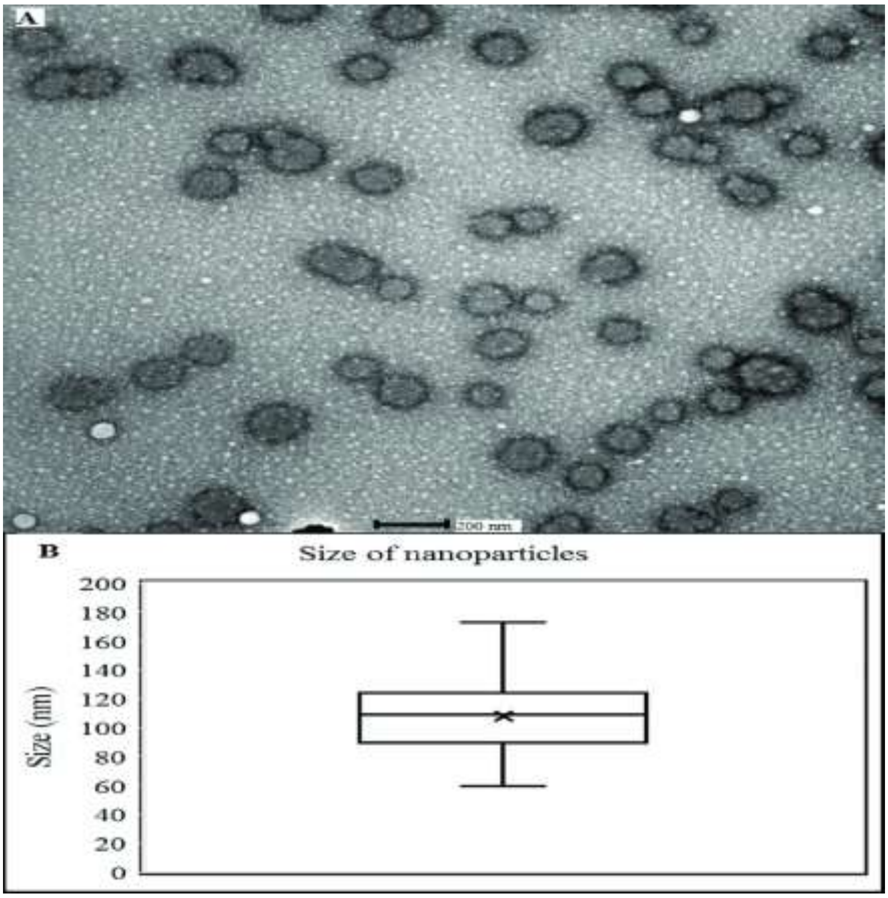

Figure 5. Transmission electron microscopy of chitosan-propolis nanoparticles: A) Chitosan-propolis nanoparticles were stained with uranyl acetate and examined by transmission electron microscopy to determine the average particle size. B) Box plot depicting the average size of the nanoparticles calculated using ImageJ software

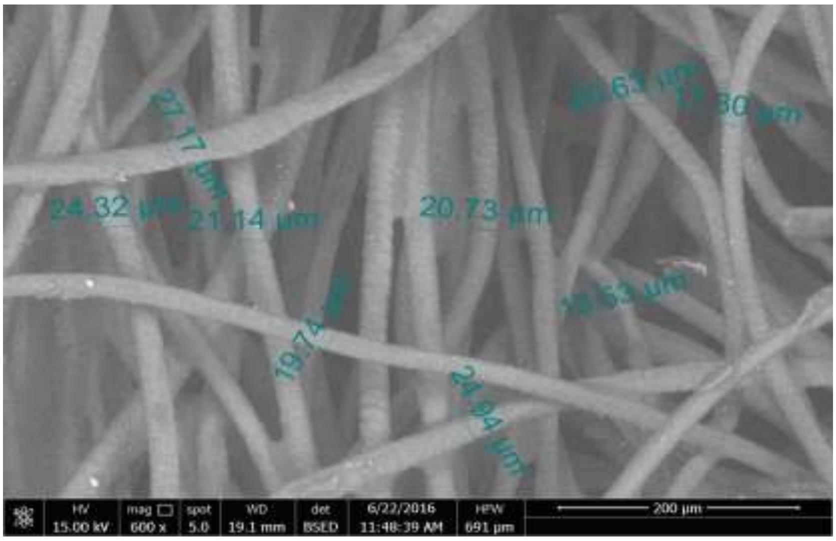

Figure 6. SEM for untreated wool fibers

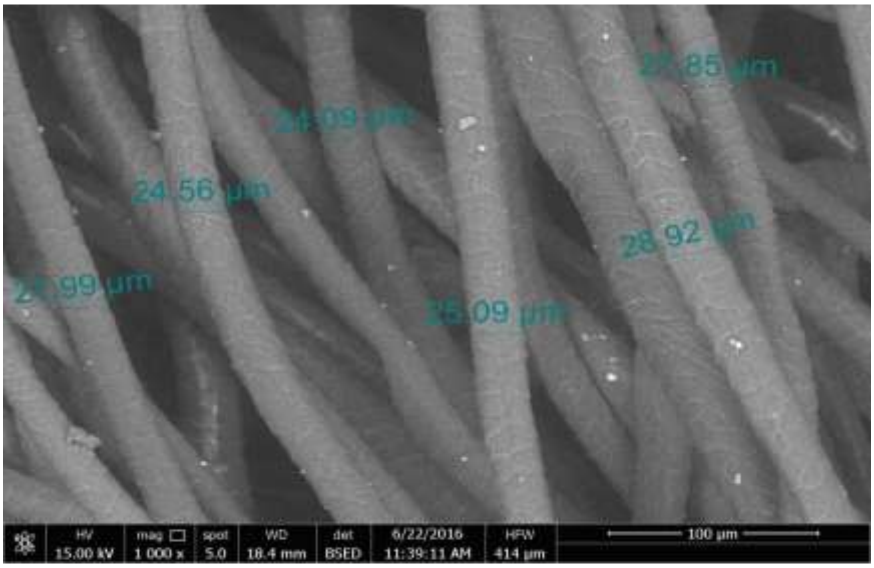

Figure 7. SEM for pretreated wool fibers with 4g/L chitosan-propolis nano composite

Discussion

There are many hypothesis explaining the possible action of ultrasonication on the dyeing system. Theoretical explanations have been presented which attribute this effect to wave's high energy influencing the dyeing system by means of mechanical or hydrodynamic forces associated with cavitation which affected on the structure of dye (Ali *et al.,* 2019). Chitosan - propolis nano composite pretreatment of wool fibers led to high dye uptake. Color strength (K/S) exhibited high values due to the treatment. The fastness properties including light, washing, and perspiration of dyed fibers were increased due to the treatment of wool fibers by the prepared nano composite. The dye extraction and color strength (K/S) of wool fibers indicated that microwave heating is more effective than ultrasonication. Microwave is economical method, it saves time and energy. It is also eco-friendly and produces a higher dye uptake as compared to ultrasonication technique. The effect of microwave heating mechanism is through ionic conduction, which is a type of resistance heating. Depending on the acceleration of the ions through the dye solution, it results in collision of dye molecules with the molecules of the fiber.

Natural dyes are non-polluting, which involve inexpensive equipment and small-scale operations. It was documented that propolis has different antimicrobial mechanisms, including inhibition of cell division, collapses microbial cytoplasm cell membranes and cell walls, inhibition of bacterial motility, enzyme inactivation, bacteriolysis, and protein synthesis inhibition (Fernandes Júnior, *et al.,* 2005, Takaisi and Schilcher, 1994). The polyphenols of propolis interacts with many microbial proteins by forming hydrogen and ionic bonds, thus altering the three-dimensional (3D) structure of the protein and as a consequence affects its functionality (Wink, 2008, Wink, 2015).

The antimicrobial activity towards some species of bacteria and a fungus was also determined and the results obtained showed high percentage reduction for the treated fibers than the untreated ones. The highest records of reduction of microbes (antimicrobial activities) were obtained at high concentrations 2 and 2.5g/L of chitosan-propolis nano composite Antimicrobial activity varies by varying the strains of bacteria and fungi, the bacterial reduction rate increases as the concentration of chitosan/propolis composite increases. The degree of these increase depend on the kind of bacteria and fungi (Ali *et al*., 2015; El–Mohamedy and Aboelfetoh, 2014 a,b). Antimicrobial activities of both wool fibers treated with natural dye and the investigated composite treatments were more effectiveness against *Staphylococcus aureus* G+ than *Escherichia coli* G- as well as pathogenic fungus *Aspergillus niger*. The results showed that the composite exhibited antimicrobial activity of treated fibers were higher than the untreated ones. There was a slight difference in antibacterial activity between wool fibers dyed with microwave and ultrasonication method. Many investigators reported that many natural dyes combined with chitosan-propolis nano composite treatment have antimicrobial activity against pathogenic bacteria and fungus. The antibacterial activity of the prepared sample fabrics against Gram-positive (*S. aureus*) is greater than the antibacterial activity against Gram-negative (*E. coli*), most probably due to the fact that Gram-positive bacterial cell walls consists of a single layer, whereas Gram-negative cell wall is a multilayered structure bounded by an outer cell membrane (Ali Shtayeh *et al.*, 1998).

It concluded that wool fibers pretreated with the tested nanomaterials exhibited better results than the untreated samples. Nano composite of chitosan and propolis showed antimicrobial activity against some pathogenic fungus and bacteria. The results indicated that the antimicrobial activity of the natural dye under investigation was good and it was enhanced by treatment with the nano composite. The morphological structure of the untreated and pretreated wool fibers was examined by scanning electron microscopy. The untreated wool fibers have a rough surface. The pretreated wool fibers were swollen as compared to the untreated fibers. The diameter of the fibers increased, and the surfaces became smooth and even.

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References

Ali, N. F., El-Khatib, E. M. and El-Mohamedy, R. S. R. (2015). The antimicrobial activity of pretreated silk fabrics dyed with natural dye. International Journal of Current Microbiology and Applied Sciences, 4:1166-1173.

Ali, N. F., El-Khatib, E. M., El-Mohamedy, R. S. R, Nassar, S. H. and El-Shemy, N. S. (2019). Dyeing properties of wool fibers dyed with rhubarb as natural dye via ultrasonic and conventional methods. Egyptian Journal of Chemistry, 62:119-130.

Ali, N. F., Mohamedy, R. S. R. E. L. and El- Khatib, E. M. (2011). Antimicrobial Activity of Wool Fabric Dye with Natural Dyes. Research Journal of Textile and Apperal, 15:1-10.

Ali-Shtayeha, M. S., Reem, Yaghmour, M. R., Faidi, Y. R., Salem, K. and Al-Nurid, M.A. (1998). Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. Journal of Ethnopharmacology, 60:265-271.

Ben-David, A. and Davidson, C. E. (2014). Estimation method for serial dilution experiments. Journal of Microbiological Methods, 107:214-221.

Dutta, P. K., Ravikumar, M. N. V. and Dutta, J. (2002). Chitin and chitosan for versatile applications. Journal of Macromolecular Science Part C – Polymer Reviews, 42:307-354.

El–Mohamedy, R. S. R. and Aboelfetoh, M. A. (2014a). Evaluation of antifungal activity of Moringa oleifera extracts as natural fungicide against some plant pathogenic fungi Invitro. Journal of Agricultural Technology, 10:963-982.

El–Mohamedy, R. S. R. and Aboelfetoh, M. A. (2014b). Antifungal activity of Moringa oleifera oil and seed extract against some plant pathogenic fungi. Middle East Journal of Agriculture Research, 3:242-249.

Fernandes Júnior, A., Balestrin, E. C., Betoni, J. E. C., Orsi, R. D. O., Cunha, M. D. L. R. D. and Montelli, A. C. (2005). Propolis: Anti-Staphylococcus aureus activity and synergism with antimicrobial drugs. Memórias do Instituto Oswaldo Cruz, 100:563-566.

Koukaras, E. N., Papadimitriou, S.A., Bikiaris, D.N. and Froudakis, G. E. (2012). Insight on the Formation of Chitosan Nanoparticles through Ionotropic Gelation with Tripolyphosphate. Molecular Pharmaceutics, 9:2856-2862.

Shin, Y., Yoo, D. I. and JANG, J. (2010). Molecular weight effect on antimicrobial activity of chitosan treated cotton fabrics. Journal of Applied Polymer Science, 80:2495-2591.

Simone-Finstrom, M., Borba, R. S., Wilson, M. and Spivak, M. (2017). Propolis counteracts some threats to honey bee health. Insects, 8:46.

Takaisi-Kikuni, N. B. and Schilcher, H. (1994). Electron microscopic and microcalorimetric investigations of the possible mechanism of the antibacterial action of a defined propolis provenance. Planta Medica, 60:222-227.

Wink, M. (2008). Evolutionary advantage and molecular modes of action of multi-component mixtures used inphytomedicine. Current Drug Metabolism, 9:996-1009.

Wink, M. (2015). Modes of action of herbal medicines and plant secondary metabolites. Medicines, 2:251-286.

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