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Study on the Effect of Pomegranate Peel and Walnut Green Husk Extracts on the Antibacterial and Dyeing Properties of Wool Yarn Treated by Chitosan/Ag, Chitosan/Cu Nano-particles

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ABSTRACT

The growth of microorganisms on textiles can reduce mechanical properties, color changing, and disease spreading. Hence, natural antibacterial agents have developed dramatically and are important in the textile industry. In this study, the role of pomegranate peels and walnut green husk extracts on the antibacterial, dyeing, and fastness properties of wool yarn treated with chitosan-Cu and chitosan-Ag nanoparticles (Ch-Cu NPs and Ch-Ag NPs) are investigated. It was found that pomegranate peel and walnut green husk extracts could increase the antibacterial activities (almost 100 %) of samples in lower concentrations (1 % o.w.f.) treated with Ch-Cu and Ch-Ag NPs. Furthermore, the durability of the antibacterial activity of dyed samples was acceptable after 10 repeated washing times. Ag and Cu on the wool samples provided varied shades, which can be related to form complexation/coordination between them and natural dyes. The obtained results clearly showed that the extracts of used natural dyes could be proposed as the green method for decreasing the consumption of Ch-NPs, consequently, the costs of antibacterial finishing. Prog. Color Colorants Coat. 16 (2023), 221-229© Institute for Color Science and Technology.

1. Introduction

Research and development about natural antibacterial agents in the textile industry have attracted the attention of researchers [1, 2]. Many antibacterial agents such as copper naphthenate and numerous mercury compounds and formalin have been utilized in former that are nowadays firmly banned owing to their hazardous and toxicity potential for ecological [3, 4]. Metallic salts or oxides as alternate antibacterial agents have been used in textiles, but their danger to humans and aquatic life is still a problem [5, 6]. Also, the bioactivity of these materials on textiles is reduced substantially; therefore, more amounts are required to preserve their property [7]. The trend of research indicates that natural antibacterial agents have become more widely used. Various natural sources may be used as an alternative to conventional antibacterial agents for dyeing and finishing textiles [8, 9]. Simultaneous dyeing and finishing of textiles by natural antibacterial dyes can reduce production costs, water effluent, and environmental problems.

The walnut (*Juglans regia L.*) tree is cultivated in different regions of the world, and in terms of food, it has a very high consumption and is useful [10, 11]. 5-hydroxy-1,4-naphthoquinone is present in the fruits and leaves of this plant. This compound, with the original name C.I. Natural Brown 7, is a natural dye that can be used for dyeing of textiles [12, 13]. Many papers have

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been reported about the extraction, dyeing, and different uses of a walnut shell. Various fibers can be dyed with this dye, and antibacterial and antioxidant activities have also been described in extracted species from this plant owing to high phenolic contents [14, 15].

Pomegranate is a small tree named (*Punica granatum*) in the family *Lythraceae*, a native plant of Iran and nearby regions, and its height varies between 5 and 10 meters. Pomegranate peel is a waste of fruit juice processing. They can be used as natural colorants with antioxidant, anticarcinogenic, and antibacterial activities due to the presence of flavonoids and polyphenols and alkaloids such as N-methyl granatonin [16, 17]. Pomegranate peel has various uses in different fields and textile dyeing. Also, different textiles can be dyed with the antibacterial and antioxidant activities of pomegranate peel [18-20].

Chitosan, a biopolymer, is obtained from chitin as the second natural polysaccharide after cellulose [21, 22]. The unique properties of chitosan, such as antibacterial, biodegradability, and non-toxicity, have led to the development of its various applications. It is possible to enhance the inherent properties of chitosan by grafting different metal/metal oxides and generating novel functionalities on it [23, 24]. The antibacterial activity of chitosan can improve 10-20-fold by metals, so silver ions increase the antibacterial activity of chitosan up to 20-fold other than chitosan [25].

Natural dyes are green materials that increase the antibacterial efficiency of chitosan on textile fibers [26, 27]. Shin et al. reported that the dye uptake and antibacterial activity were increased with the increase in chitosan concentration. Chitosan pretreatment effectively develops eco-friendly, functional, natural-dyed blend fabrics [28]. The cotton fabric successfully printed with natural dyes in fine powder form using chitosan as an eco-friendly mordant [29]. The chitosan showed good colorimetric data and antibacterial activity in the printed samples [30]. The combination of chitosan and henna dye was studied by Bhuiyan et al. They suggested that the antibacterial activity of jute fiber against the bacteria can considerably improve by chitosan and henna dye [31]. These outcomes recommend that the use of chitosan and natural dye from henna onto jute fiber is an approach to get the desired dyeing and antibacterial property.

In this study, the treated samples with Ch loaded by Cu and Ag ions were dyed with extracts of walnut green husks and pomegranate peels as two natural dyes. Moreover, the antibacterial activities, durability, colorimetric data, and color fastness of treated samples against wash and light were investigated.

2. Experimental

2.1. Material and methods

$AgNO_3$ and Cu_2O were purchased from Merck Co. (Germany). Mahtani Chitosan PVT LTD Chitin India supplied chitosan with molecular weight>600kD and a degree of deacetylation of 98 %. The walnut green husks and pomegranate peels were provided from a local dyeing workshop in Ardakan (Iran). The chemical structures of colorants in the mentioned dyes are given in Figure 1. The wool yarns with the specification of 200Tex/4fold were scoured with nonionic detergent (Lotensol, Hansa). The other solvents and chemicals were analytical grade and utilized without any purification.

2.2. Wool treatment

The synthesis, characterization, and application of Ch-Cu and Ch-Ag NPs on the wool yarns have been reported in detail in our previous paper [32, 33]. Briefly, various concentrations of $AgNO_3$ and Cu_2O salts (1, 5, and 10 % o.w.f.) were prepared in 30 mL water containing citric acid (4 % o.w.f.) and sodium hypophosphite (4 % o.w.f.) at pH 4. Then, the wool sample (1 g) was added to the prepared solution and was shaken at 120 rpm at 50 °C for 2 h. Finally, the samples were rinsed and dried at room temperature.

2.3. Antibacterial test

A quantitative test AATCC 100-2004, was used to evaluate the bacterial reduction of dyed samples against gram-positive *S.aureus* and gram-negative *E.coli* bacteria. In summary, samples were autoclaved at 121 °C for 15 min. 100 µL of prepared suspension of bacteria (10^6 bacteria) was added into 10 mL prepared nutrient broth and incubated at 37±2 °C for 18 h. The reduction percent in the number of bacterial colonies of the wool samples was calculated by Eq. 1:

$$\textit{Antibacterial effect (\%)} = \frac{A - B}{A} \times 100 \qquad (1)$$

Where A is the number of bacteria colonies in the solution containing the wool sample, and B is the number of bacteria colonies in the solution containing the dyed samples.

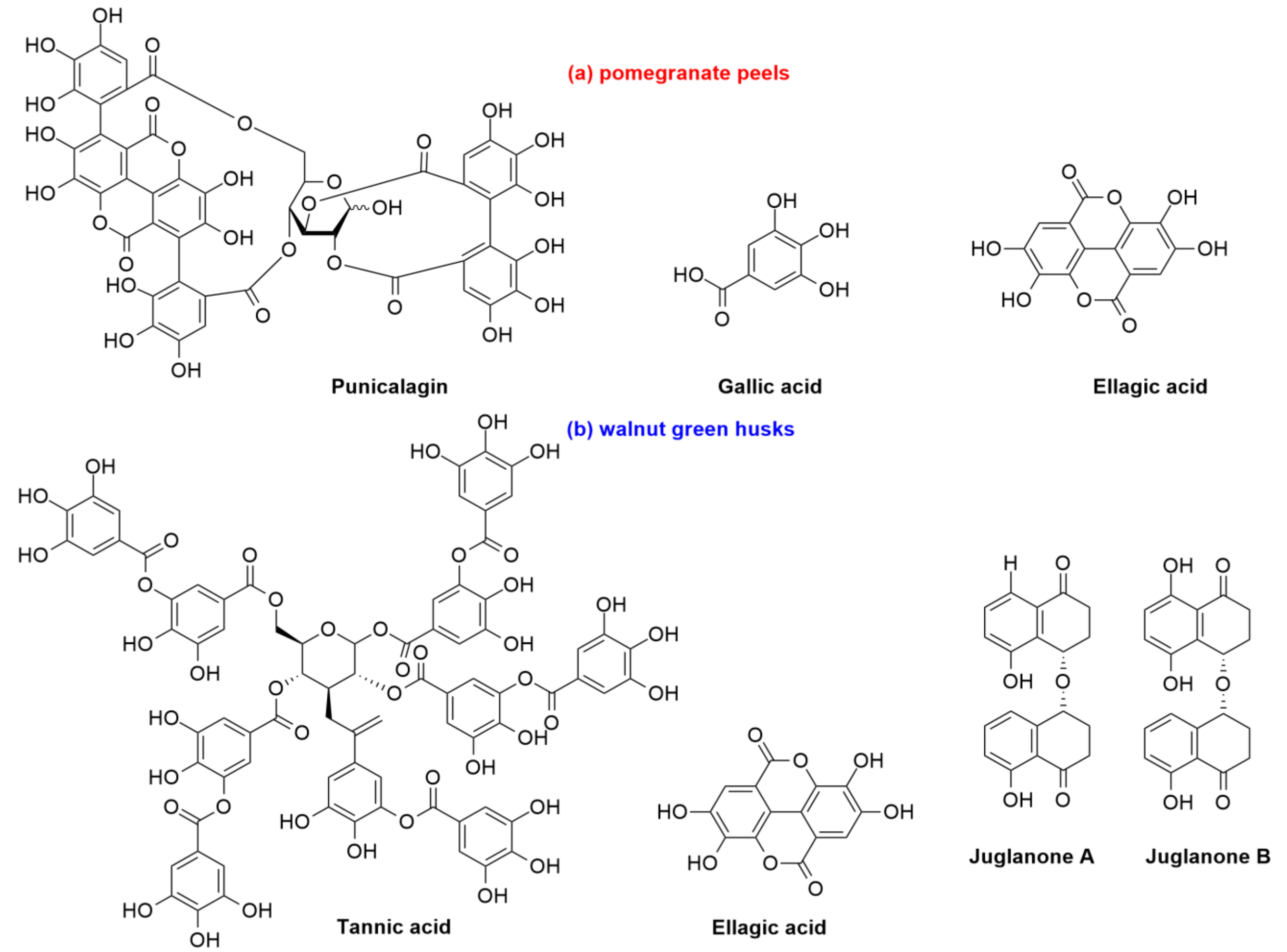


Figure 1: The chemical structure of colorants in the (a) pomegranate peels and (b) walnut green husks.

2.4. Dyeing procedure

The walnut green husks and pomegranate peels were extracted in 30 % o.w.f. Concentration through the conventional method at boiling temperature for 2h. All samples were dyed with acetic acid at 100 °C for 60 minutes at a liquor ratio of 1:40, at a pH of 5 [32]. Finally, dyed samples were washed and dried.

A reflective spectrophotometer used to determine the colorimetric analysis of dyed samples through the Kubelka-Munk function (Eq. 2).

$$\frac{K}{S} = \frac{(1-R)^2}{2R} \quad (2)$$

Where *K* and *S* are the absorbance and scattering coefficients, respectively. *R* is the reflectance ratio at maximum absorbance wavelength. The measurements were performed at three places on the sample, and their averages were reported.

2.5. Color fastness

The wash fastness of dyed samples was determined by ISO 105 C06 C2S:1994 (E) manner. The dyed samples were placed between a piece of undyed wool and cotton adjacent fabrics so that three samples were stitched together to form composite specimens. The samples were washed at 60 °C for 30 min, then rinsed and dried at room temperature. The grey scales were used for measurements. The rates of 1 and 5 indicate severe staining and no staining on adjacent fabrics, respectively.

The xenon arc lamp considered the fastness to light of dyed samples via the standard ISO 105 B02:1988 (E). The samples and eight standard patterns of the blue wool fabric of various light fastness ratings were placed inside the light fastness tester. The samples were evaluated after 100 h of exposure to a xenon lamp so that the rates of 1 and 8 indicate weak and excellent light fastness of dyed samples, respectively.

3. Results and Discussion

3.1. FTIR analysis

Figure 2 shows the FTIR spectra of natural dyes and untreated, treated wool fibers. In Figure 2a, the O-H stretching and C=C or C=O functional groups of polyphenols in natural dyes were observable in the range of 3300 cm^{-1} and 1630-1640 cm^{-1}, respectively [28, 30]. Also, a peak at 1024-1041 cm^{-1} corresponds to C-O-C vibration in natural dyes [32, 33]. Colorants available in pomegranate peel and walnut husk extracts are shown in Figure 1. Many -O.H., C=O, -C-C-, -C-H- bonds, and -C=C- aromatic rings are visible in the spectra. The FTIR spectra showed that the obtained data are consistent with the previously reported results [14, 26].

FTIR spectroscopy of raw and treated wool samples is shown in Figure 2b. Results showed a strong peak at 3400 cm^{-1}, related to the wool's different amines and hydroxyl functional groups. The C-H stretching vibration peak at 2923 cm^{-1} confirmed the presence of -CH_2 groups of wool samples. The polypeptides and proteins have various amide peaks, usually 1600-1700 cm^{-1} [26, 28]. Since the functional groups in chitosan and woolen yarn are similar and the amount of Ch-Ag and Ch-Cu NPs used is insignificant (5 % o.w.f.), the FTIR spectrum of treated samples is not much different from raw woolen yarn. However, the difference in the intensity of the peak and the presence of new peaks in the spectra of the treated wool sample can indicate the presence of Ch-Ag and Ch-Cu NPs on the wool yarn.

3.2. Antibacterial activity of wool yarn

The effects of natural dye extracts and Ch-NPs on the antibacterial activity of wool samples are shown in Table 1. Results indicated that bacterial growth was significantly inhibited as the concentration of Ch-NPs increased. Since both chitosan and NPs are each antibacterial alone, Ch-NPs hybrids have an acceptable antibacterial activity, which can be related to disrupting the nutrient to cells via interactions of cell membranes with the positively charged of Ch-NPs producing the reactive oxygen species (ROS). Consequently, the growth of the cells stops [33-35]. The highest antibacterial activity belongs to treated wool yarns with Ch-Ag, and the higher amounts are attained by increasing Ch-NPs. Results indicated that suitable antibacterial activities were obtained in 5 % o.w.f. of Ch-NPs, and this concentration was chosen as the optimal amount for wool modification.

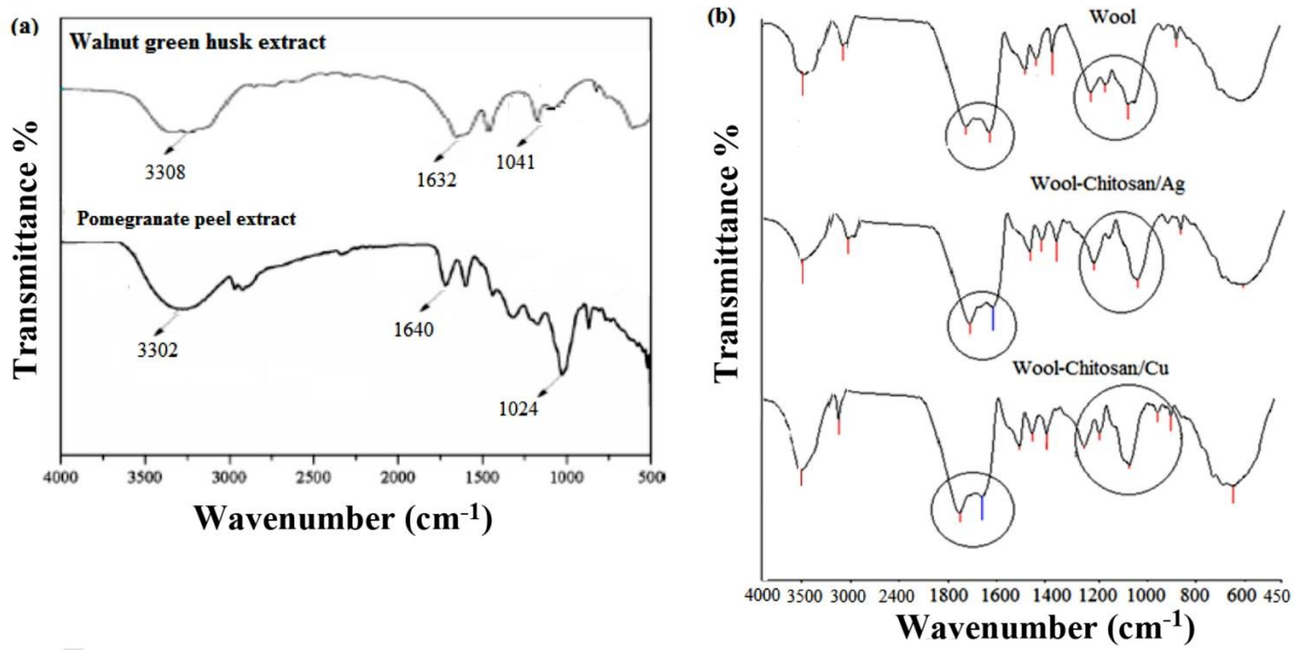


Figure 2: The FTIR spectra of (a) natural dyes and (b) untreated and treated wool samples.

Table 1: Antibacterial activity of wool samples treated by Ch-NPs at various concentrations.

Sample	%(o.w.f.)	Antibacterial activity (%)					
		Un-dyed samples		Walnut		Pomegranate	
		E. coli	*S.aureus*	*E. coli*	*S.aureus*	*E. coli*	*S.aureus*
Wool	-	-	-	63.33	55.19	70.65	64.72
Wool-Ch -Ag	1	36.11	32.08	80.40	74.17	87.68	81.41
	5	100	100	100	100	100	100
	10	100	100	100	100	100	100
Wool-Ch -Cu	1	74.61	71.81	79.27	72.38	80.16	75.32
	5	75.04	71.70	100	100	100	100
	10	80.90	74.63	100	100	100	100

Because of high ellagitannins, polyphenols, gallic acid, ellagic acid, and some flavonoids, walnuts, and Pomegranates have good antibacterial activity [14, 15, 19, 20]. Results indicated that dyeing of wool-treated samples with Walnut and Pomegranate improved the reduction of bacteria to 63 and 70 % against *E. Coli* bacterium, respectively. The obtained data against *E. Coli* bacterium were relatively better than *S. aureus* because the cell wall structures of the two bacteria are diverse, and *E. Coli* has an outer layer comprising lipopolysaccharide, which inhibits the diffusion of the antibacterial agent via the cell wall. Therefore, the most natural sources hardly kill the external membrane of the gram-negative bacterium [15, 20]. Results indicate that the dyeing of wool yarn with both natural dyes can reach higher antibacterial activities by decreasing the consumption of Ch-Cu NPs. It is possible to produce antibacterial wool yarn via a cheaper and greener process.

In Table 2, the antibacterial of wool samples was studied against repeated washing via ISO 6330-1984 standard. The washing durability of Ch-NPs on the wool fibers is affected by the strength of bonding between them. The samples dyed with walnut green husks and pomegranate peels and treated with Ch-NPs exhibited less than 10 % decrease in antibacterial activity after 10 repeated washing cycles. Furthermore, the antibacterial values of undyed samples diminished to 5-25 % after repeated washing cycles. Results showed that the antibacterial activity of dyed samples with natural dyes improved against repeated washing times, which can be related to the more and stronger linkages between the Ch-NPs-natural dyes-wool yarn systems [35, 36]. The antibacterial activities were reduced by ~ 8 % and ~25 % when Ch-Ag and Ch-Cu were used, respectively. The citric acid as a cross-linking agent in the grafting process can improve the durability of Ch-NPs on the wool samples, and the antibacterial activity was preserved. Indeed, the antibacterial activities can reduce without using citric acid [15].

Table 2: The antibacterial durability of treated samples against repeated washings.

Sample	Repeated Washing Cycles	Antibacterial activity (%)					
		Un-dyed samples		Dyed with *Walnut*		Dyed with pomegranate	
		E. coli	*S.aureus*	*E. coli*	*S.aureus*	*E. coli*	*S.aureus*
Ch-Ag	-	100	100	100	100	100	100
	5	98.40	97.31	98.40	97.31	98.51	97.06
	10	95.60	92.57	95.60	92.57	94.67	93.42
Ch-Cu	-	100	100	100	100	100	100
	5	91.81	88.80	91.81	88.80	93.06	88.90
	10	86.36	82.23	86.36	82.23	92.77	84.54

3.3. The color strength and coordinates

The colorimetric data of dyed samples with two natural dyes and treated with the Ch-NPs are shown in Table 3. a* and b* of all samples have been changed after dyeing with walnut and pomegranate. So, some samples have a different hue. The large color difference (ΔE^*) confirmed the obtained results and indicated that the treatment was effective on the final color of the samples. The color change of the dyed samples shows that a complexation/coordination bond has been established between the dye and the wool treated with Ch-NPs [37, 38]. So, it can be mentioned that no significant variation was observed in K/S values of dyed samples via these treatments, but it was possible to produce the different hues on the wool samples (Table 4). The proposed mechanism for the interaction of wool fiber and natural dyes is shown in Scheme 1. Ch-NPs can be attached to the wool via physical adsorption or chemical bonding. Also, various active sites in the protein chains of the wool fibers can improve the linkages of Ch-NPs on the wool. As a result, the number of dye absorptive sites on the wool increases, and more dye molecules can be absorbed into the treated samples [39, 40].

3.4. Color fastness properties

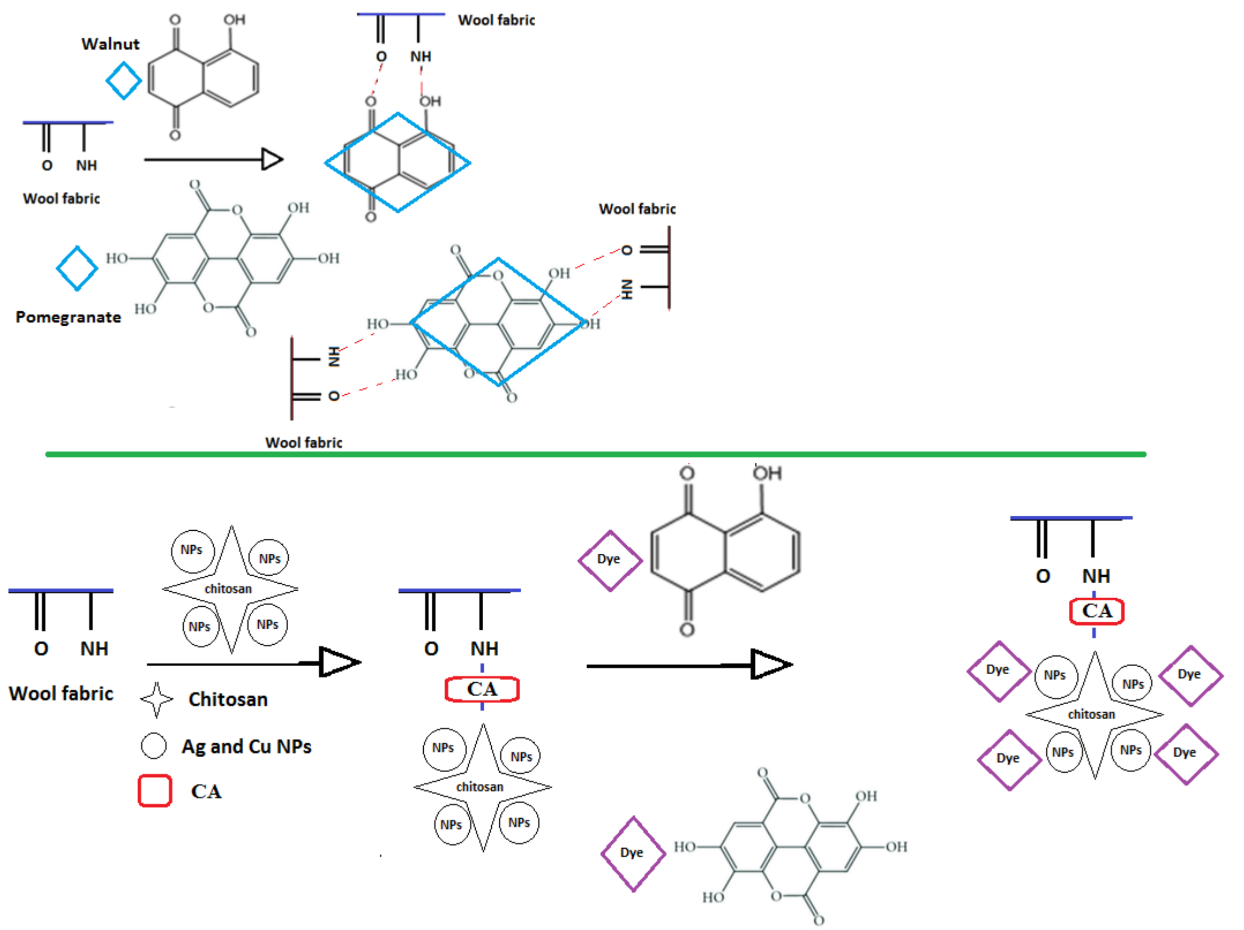
Table 4 shows the color fastness rates of dyed samples with natural dyes against wash and light. The wool dyed with both natural dyes has moderate wash fastness (3) and good light fastness (5-6). The rates of color fastness of dyed samples pretreated with Ch-NPs improved and were acceptable. So, treated samples with Ch-NPs showed excellent light fastness, which was improved to 7 rates. Also, The more linkages between natural dyes and Ch-NPs on the wool samples cause to increase in the washing fastness [38, 40].

Table 3: Colorimetric data of dyed samples with natural dyes and treated by Ch-NPs concentration 5 % o.w.f.

Dye	treated with	Before dyeing process					After dyeing process				
		L^*	a^*	b^*	K/S	ΔE^*	L^*	a^*	b^*	K/S	ΔE^*
Walnut	-	37.65	8.32	17.78	11.216	-	37.65	8.32	17.78	11.216	-
	Ch-Ag	33.07	9.34	17.743	15.820	24.24	30.85	9.8	19.523	16.690	16.27
	Ch-Cu	32.42	9.42	17.776	16.860	29.02	27.90	9.143	17.86	24.280	8.38
Pomegranate	-	62.82	5.91	17.78	7.785	-	62.82	5.91	17.78	7.785	-
	Ch-Ag	40.22	5.977	21.697	12.510	12.64	56.69	7.1	26.07	13.070	7.44
	Ch-Cu	55.16	5.726	24.906	11.400	14.88	56.41	7.58	27.036	13.650	7.86

Table 4: Colorfastness of dyed samples and treated with 5 % o.w.f. of Ch-NPs.

Dye	Sample	Treatment before dyeing				Treatment after dyeing			
		Light fastness	Wash fastness			Light fastness	Wash fastness		
			CC*	SW*	SC*		CC*	SW*	SC*
Walnut	-	5-6	3	4	4	5-6	4	4	4
	Ch-Ag	8	5-4	5-4	5-4	7	4	5-4	5-4
	Ch-Cu	7-8	5-4	4-5	4-5	7	4-5	4-5	5
Pomegranate	-	6	3-4	4	4-5	6	4	4	5
	Ch-Ag	6	5-4	5	5-4	6-5	4	5-4	4
	Ch-Cu	6	4-5	4-5	4-5	6-5	4	4-5	4-5



Scheme 1: The proposed mechanism for the interaction of wool fiber and natural dyes.

4. Conclusions

The antibacterial wool yarn was produced via an eco-friendly method using extracts of walnut green husks and pomegranate peels and Ch-Ag and Ch-Cu NPs. The wool yarns treated with Ch-NPs increase the inhibition of bacteria. The antibacterial activity of treated samples with Ch-Ag NPs and dyed by walnut husk was above 95 % against *E.coli* after 10 repeated washing times. In addition to producing acceptable antimicrobial activity on the wool, this treatment improves the color absorption of pomegranate peel and walnut husk extract by up to 6 and 13 units, respectively. It may be due to the increase in the number of absorptive dye sites on the wool, and consequently, more dye molecules can be absorbed in the treated samples. The color fastness properties of wool samples were enhanced after treatment with Ch-NPs, which can be related to the more and stronger linkages between natural dyes-Ch-NPs-wool fibers system. This study's results clearly showed that walnut green husks and pomegranate peels could raise the antibacterial activity of wool yarns treated by Ch-NPs So, the antibacterial of the sample was treated with 10 % o.w.f. Ch-NPs was 70-80 %, while natural dye extracts can increase the antibacterial; activities up to 100 % at the concentration of 5 % o.w.f. of Ch-NPs. So, the consumption of Ch-NPs for antibacterial finishing is reduced, and the antibacterial finishing of the wool yarns can be performed via an economical and green technique.

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