

Sustainable application of natural dyes on wool fibers pretreated with Chitosan/Propolis Nano composite and its antimicrobial Characteristic

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Abstract

Natural dye derived from madder root extract was used for dyeing wool because it is eco-friendly and non toxic.. The wool fibers were treated with chitosan/propolis nano composite, before being colored with a natural madder dye using microwave and ultrasonic methods . The fastness properties, color strength, color data was measured for treated and untreated fibers. and the results indicated that the treated fibers exhibited higher results than the untreated fibers. Dye concentration, time of dyeing and pH factors of the dyed wool fibres were investigated. The results showed that wool fibres treated with the tested composite exhibited higher results than the untreated.. Chitosan and propolis nanocomposites exhibit antibacterial properties against some dangerous pathogens. The results showed that the morphologies and microstructure of the treated and untreated wool fibers were examined using scanning electron microscopy (SEM). The surface of the raw wool fibers is rough. The treated wool fibers swelled in comparison to the untreated fibers. The diameter of the fibers increased due to treatment. The antimicrobial activity of the natural dye under examination was good, and the antibacterial activity of the natural dyes under study was improved by the composite. The aim of this study is the production of smart textile fibers used for manufacture of hand loom carpet.

Key words Nano composite, madder, natural dye, , dyeing antimicrobial activity

Introduction

Both pathogenic and non-pathogenic microorganisms are frequently found in human epidermis, nasal cavities, and other areas like the genital area. Microorganisms can enter a textile material directly through clothing or other textiles. It is among the most likely reasons why hospital infections occur. According to recent research, contaminated objects have the potential to disperse germs into the surrounding area. Textiles have been discovered to contain pathogenic microorganisms, including *Pseudomonas aeruginosa*, *Candida albicans*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* [1]. Microbes can also result in stains, unpleasant smells, and damage to the mechanical characteristics of fibers, all of which can reduce the effectiveness of a product used in the textile industry.. Antimicrobial textiles can significantly decrease environmental pollution and human infection [2].

Chemicals that stop bacteria from growing are known as antimicrobial agents, and they can be synthetic or natural. They can either prevent the production of proteins, lipids, or enzymes—all necessary for cell survival—or they can kill the microorganisms by disintegrating their cell walls. Biocides make up the majority of synthetic antibacterial agents used on textiles. Antimicrobial fabrics can be used to make a wide range of products, such as athletics, outdoor apparel, undergarments, shoes, furniture, hospital linens, wound care wraps, towels, and wipes. Many testing techniques have been established to evaluate the effectiveness of antimicrobial fabrics, such as the agar diffusion test (a qualitative method like the halo method) and the dynamic shake test (a quantitative technique like the serial dilution and plate count method). [3]. Bees use a variety of plant materials, including leaves, flowers, and bud exudates, to make propolis, often known as bee glue. Wax and bee secretions alter propolis [4,5].

Numerous research teams have shown that different forms of propolis have antimicrobial qualities. demonstrated the antibacterial properties of poplar propolis against a variety of Gram-positive and Gram-negative bacteria, including isolates of *Staphylococcus aureus* that are resistant to the drug methicillin (MRSA) [6]. The purpose of this study is to prepare wool fibers with propolis to enhance their coloration and antimicrobial properties. It is essential to avoid cross-infection by dangerous microorganisms. The microbial invasion is controlled by these treatments. They also inhibit the metabolism of bacteria to halt the formation of odors, discolorations, stains, and deterioration of the fiber products.

However, using a natural dye made from madder is the aim of this study. In an attempt to lessen industrial pollution and safeguard the environment, textiles have been colored using natural dyes, appealing in a manner akin to that.

Plant description:

Rubia cardifolia(madder)

Systematic position

Kingdom. Plantae (plant)

Phylum.Tracheophyta vascular plant)

Class: Magnoliopsida (Dicot.)

Order: Gentianales (Rubiales)

Fanily: Rubiaceae

Genus. Rubia

Rubia cordifolia, known as **Indian madder**, is a species of flowering plant in the coffee family, Rubiaceae. It has been cultivated for a red pigment derived from roots.

2-.Material and method

2.1.Materials

2.1.1.Madder roots were collected from Research Agriculture Institute in Egypt In compliance with relevant institutional, national, and international laws and regulations, plant roots were collected. After thoroughly cleaning them with distilled water to remove any impurities, they were let to dry at room temperature. A laboratory-scale mill (M 20; IKA, Staufen, Germany) was used to grind the dried roots.[6].

2.1.2.wool fibers 10/2, provided by the Egyptian El Mahalla compny.

2.1.3.Propolis.and chitosan

2.2Appliances: Ultrasonic: A 5.75-liter thermostatic CREST desktop 575 HT ultrasonic cleaner with a maximum output of 500 Watts and a frequency of 38.5 kHz was used. The output power is between 500 and 100 watts.

Microwave: The device used in this experiment was the Samsung M 245 microwave, which had a 1,550 watt output and ran at 2450 MHz.

Extraction of natural coloring matter

Madder were crushed to the powder form, and then the coloring matter was extracted using (20-80 g of the powder in 1000 ml water) at the boiling for one hour. At the end, the solution was filtered off and left to cool down.[6].

2.3.Propolis Extraction

Propolis was extracted using a variety of solvents, including ethanol, water, methanol, and ethyl acetate. For the majority of them, ethanol extracts have the highest activity. Soxhlet extraction with ethanol and maceration are easy methods for extracting propolis. Ethanol extraction is useful for producing propolis extracts that are high in medicinal components and low in wax. Additionally, if propolis extracts are utilized as dietary supplements, ethanol is quickly eliminated and non-toxic. [7–8- 9]

2.4.Treatment of wool fibers using chitosan/propolis composite

Wool fibers are treated using pad-dry-cure techniques. The composite (2–10%) was applied to the wool fibers, padded to 100% wet pick up, dried for five minutes at 80 °C, and then cured for three minutes at 120 C⁰ [10].

SEM stands for scanning electron microscopy. The surface morphology of treated and untreated wool fibers was examined using a JSMT-20 scanning electron microscope (JEOL-Japan). Before inspection, the surface of the wool fibers was prepared on a suitable disk and periodically sprayed with gold. The SEM experiment was conducted at the National Research Center in Egypt.

2.5.Dyeing procedures:

2,6. Microwave dyeing of wool fibers

Wool fibers were microwave-heated at various pH levels (3–9) for varying durations (1–5 minutes) in a dye solution with varying dye concentrations and a 30:1 liquor ratio. Following a 30-minute soak in a 60:1 liquor ratio and 3 g/L non-ionic detergent (Hostapal CV, Clariant), the dyed samples were rinsed with cold water and allowed to dry at room temperature before being rinsed again.[11].

.2.7.Wool fibres dyed using an Ultrasonic technique

Wool fibers were ultrasonically colored with two grams of Madder natural dye at pH 5 and L.R. 1:50. The samples were dyed for 30 minutes at 50 degrees Celsius using a 500 watt ultrasonic power source[12]. They were rinsed with cold water and then immersed for 30 minutes at 50°C in a solution containing (5 g) of non-ionic detergent. They were then cleaned and allowed to air dry at room temperature.

2.8.Colorimetric measurements of data

All colored materials' color coordinates and color strength (K/S) were specified using the CIELAB color space system, which is occasionally written as L*, a*, and b* coordinates. A Hunter Lab UltraScan PRO spectrophotometer (USA) fitted with an illuminant D65, 10 standard observer was also used to assess the following parameters: b* signifies yellowness if the sample is positive, and blueness if it is negative; c* indicates chroma; h represents hue angle; and v* indicates lightness or darkness (a higher lightness value indicates a lower color yield).The color difference values (ΔE^*) were calculated [11].

2.9.Assessments of the antimicrobial activity

The antibacterial properties of wool fibers treated with propolis composite and dyed with madder natural dye. The colors were assessed using standard techniques such as the serial dilution and plate count procedures.

The serial dilution blanks were autoclave sterilized after being made in bottles with 99 milliliters of distilled water and consecutively labeled with numbers ranging from 10⁻¹ to 10⁻⁵. By adding 1.0 gram increments to a 99 ml test tube labeled 10⁻², or the 10⁻² dilution, each fiber sample was diluted 10⁻¹; this process was repeated for each level up to 10⁻⁵.Bacterial strains were counted using peptone of food quality Agar medium, while fungal strains were counted using potato dextrose agar (PDA) medium. The conventional spread plate technique was used to distribute 0.1 ml of dilution fluid from 10⁻³, 10⁻⁴, and 10⁻⁵ dilution tubes in triplicate on sterile petriplates in order to isolate bacterial and fungal strains. After that,

the PDA plates were incubated for 72 hours at 27 °C and the LB agar plates for 24 hours at 37 °C. Once the microorganisms had grown successfully, the morphologies, colors, transparency, and other features of each individual colony were identified. The bacterial cellular structure and gram response were observed using Gram staining. Colony Forming Units (CFU) were used to express the density of the fiber samples after the number of bacterial and fungal colonies was counted [12].

Samples were counted, the antimicrobial activity was calculated and expressed, and the density was expressed in Colony Forming Units (CFU). The antibacterial activity was calculated and shown as a decrease in the overall number of bacteria and fungi in each treatment.4. a decrease in the overall number of bacteria and fungus .

3-Results and discussion

3.1. Effects of different amounts of propolis and chitosan

The effects of varying propolis concentrations were examined in wool fibers manufactured with propolis and stained with madder natural dye. The findings demonstrated that when wool fibers were treated with propolis and dyed with curcumin colors, the highest value of K/S was achieved with a 2 ppm concentration of propolis. Microwave Table 1 and the ultrasonic method, as indicated in Table 2, were used to evaluate this. Ionic conduction, a form of resistive heating, was used to microwave color the fibers. The dye molecules collide with the fiber molecules as a result of the ions accelerating through the dye solution [13]. According to theoretical interpretations, this phenomenon is caused by the high wave energy affecting the dyeing system by mechanical or hydrodynamic forces connected to cavitation that alter the dye's structure.3.2.Colour data of wool fibers dyed with madder-and pretreated with the composite at varying concentrations.

Table 1 shows the colorimetric data (L^* , a^* , and b^*) of many fibers dyed with different concentrations of microwave-extracted dye. The table's data suggests that the samples' color darkened as the extracted dye concentration rose and L^* values dropped. As the dye concentration increased, so did the values of a^* and b^* . As the dye concentration rose from 0.5 to 2 ppm, the color of the colored wool fibers deteriorated and turned more reddish yellow. Table 2 shows that the same results were obtained using the ultrasonic approach.3.3. Wool color data

3.2. Fibres treated with propolis composite and dyed with madder at varying concentrations.

Table 1 displays the colorimetric data (L^* , a^* , and b^*) of various fibers that were microwave-dyed with varying concentrations of extracted dye. The information in Table indicates that as the extracted dye concentration increased, L^* values decreased and the samples' color darkened. The values of a^* and b^* improved as the dye concentration rose [14]. As the dye concentration increased from 0.5 to 2 ppm, the color of the dyed wool fibers darkened and became more reddish yellow. The identical ultrasonic technique findings are shown in Table 2.

Table 1: Colour data of wool fibres pretreated with chitosan/ prpolis and dyed with madder natural dye at different concentrations using an ultrasonic method

Conc.of chitosan/ prpolis	K/S	L^*	a^*	b^*	C^*	ΔH	ΔE
0.5	7	61.2	18.5	9	22	25	40
1.0	10.42	50.5	32	14.3	34.5	25.2	52.2
1.5	15.3	49	34.6	15.5	36	25.5	54.83
2.0	1625	41.5	36.5	16	37	26	57
Untreated	76	54	34.7	21	40.5	28.5	31

Table 2 Effect of conc. of propolis on wool fibers dyed with madder dye by microwave

Conc.of composite	K/S	L^*	a^*	b^*	C^*	ΔH	ΔE
0.5	2.51	54.4	45	4.8	43	6.5	57
1	4.1	43.5	44.24	11.65	46	13	51.3
1.5	22.87	59.8	57	23	60.4	19.5	70.6
2	7.4	41.3	51.5	13	53.5	16	64.
2.5	6	49.5	40	14	52.7	16	57

3.3.Effect of pH on the color strength of dyed wool fibers pretreated with .the composite

The pH of the dye bath affected the color strength of wool fibers colored with the natural dye under investigation. In an acidic solution, the dye has a strong affinity for the fibers. Cationized amino groups can use electrostatic attraction to adsorb anionic dye molecules in acidic environments [15]. Figure 2 illustrates that the wool fiber dye produced the highest color strength (K/S) value at pH 5 for the ultrasonic method and pH 4 for the microwave approach.

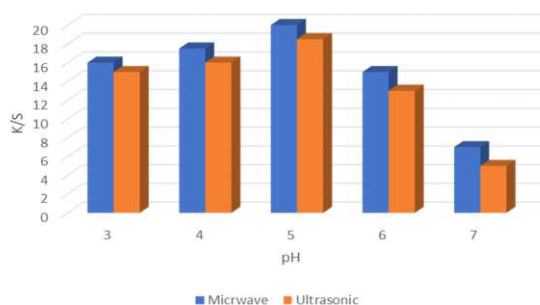


Figure 1 Effect of pH on color strength of dyed wool fibers that have been treated with propolis composite and untreated dyed fibers using ultrasonic and microwave.

3.4.Effect of time on the color strength of microwave-dyed fibers, both treated and untreated

The effects of dyeing duration on propolis-treated wool fibers that were subsequently microwave-dyed with curcumin are shown in Figure 2. The statistics indicated that the color strength reached its maximum values after five minutes.

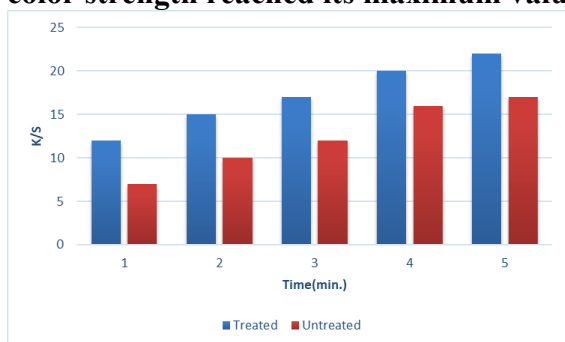


Figure 2. Effect of time of dyeing on the color strength for wool fibers pretreated with propolis and untreated dyed with madder using microwave and Ultrasonic.

3.5-Antimicrobial characteristics

Propolis has been shown to exhibit a variety of antibacterial properties, such as the ability to stop cell division, degrade microbial cytoplasmic cell membranes and cell walls, stop bacterial migration, deactivate enzymes, cause bacteriolysis, and stop protein synthesis [16].

The propolis polyphenols will create ionic and hydrogen connections with a variety of microbial proteins. Two pathogenic bacterial isolates, *Staphylococcus aureus* and *Escherichia coli*, and one pathogenic fungal isolate, *Aspergillus niger*, were used to assess the antibacterial efficacy of wool fibers dyed with curcumin. Natural dye was mixed with propolis [17].

The tables' findings demonstrate how the propolis content affects wool fibers' antibacterial activity. The antibacterial activity of various bacterial and fungal strains varies, and the quantity of propolis accelerates the rate of bacterial decline. The type of bacteria and fungus determines how much they grow [18]. Numerous studies have demonstrated that certain natural colors have antibacterial qualities against dangerous germs and fungus when combined with propolis. Compared to Gram-negative (*E.coli*) bacteria, Gram-positive (*S.aureus*) bacteria are more vulnerable to the antibacterial activity of the prepared sample fabrics. Gram-positive bacteria have single-layered cell walls, while Gram-negative bacteria have multilayered cell walls encased in an outer membrane. This is most likely the cause of the variation in antibacterial activity [19, 20]. The findings demonstrate that the treated fibers have greater antibacterial activity than the untreated fibers. The antibacterial qualities of wool colored by ultrasonic and microwave methods differ slightly. Gram-positive bacteria have single-layered cell walls, however wool fibers treated with natural dye and propolis were found to have stronger antibacterial properties than *Escherichia coli* G⁻ against dangerous fungus like *Aspergillus niger* and *Staphylococcus aureus* G⁺ [20]. This is most likely the cause of the variation in antibacterial activity [21]. The findings demonstrate that the treated fibers have greater antibacterial activity than the untreated fibers. The antimicrobial qualities of wool colored using ultrasonic and microwave methods varied slightly [15]. It was found that wool fibers treated with propolis and dyed with madder natural dye had better antibacterial properties compared to *Escherichia coli* G^{-ve} against dangerous fungi including *Aspergillus niger* and *Staphylococcus aureus* G^{+ve} [22].

Table 3. Antimicrobial activity of dyed wool fibers pre treated with different concentrations of the composite and dyed by microwave and ultrasonic methods

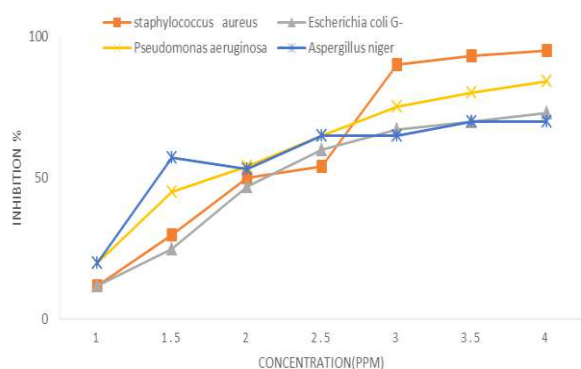
Microorganism		Growth reduction (%) of different microbes on fibers treated with chitosan at concentration (ppm)				
		0 control	1	1.5	2	2.5
<i>Staphylococcus aureus</i> G ⁺	Microwave	10	28	40	57	90
	Ultrasonic	11	20	49	53	86
<i>Pseudomonasaeruginosa</i>	Microwave	20	46	53	66	81
	Ultrasonic	17	31	40	56	76

<i>Aspergillusniger</i>	Microwave	20	56	54	67	78
	Ultrasonic	16	45	50	64	65
<i>Escherichia coli G-</i>	Microwave	12	27	48	60	77
	Ultrasonic	10	15	28	40	54

Control : untreated wool

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

Microorganism	Conc..	ppm					
	1	1.5	2.	2.5	3	3.5	4
<i>Staphylococcus aureus G+</i>	8	31	3	55	89	92	94
<i>Escherichia coli G-</i>	12.	27	46	61	66	71	74
<i>Pseudomonas aeruginosa</i>	20	45	55	64	77	45	85
<i>Aspergillus niger</i>	22	55	56	67	66	70	75



.Figure 3)Minimum inhibitory concentration of the nano composite

3.6. Surface anatomy

Using scanning electron microscopy (SEM), the morphologies and microstructure of the pretreated and untreated wool fibers were investigated. Figure 4 shows scanning electron microscopy (SEM) images of the untreated wool fibers, while Figure 5 shows images of the treated fibers. This demonstrates the effects of treating wool fibers with 4g/L conc. propolis [18,19,20]. As seen in Figures 4 and 5, the untreated samples have a rough surface. The treated samples had bigger fiber diameters, smooth, flat surfaces, and swelling compared to the untreated fibers. the alterations in surface morphology brought about by the treatment's active components compared to the untreated [21]

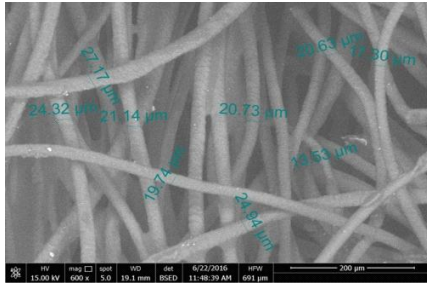


Figure 4 : SEM for untreated wool fibers.

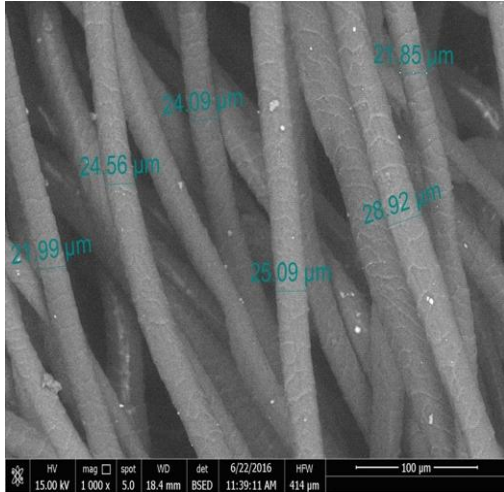


Figure 5: SEM for pretreated wool fibers with 4g/L propolis composite

4.Conclusion

Before being dyed with madder, wool fibres are treated with chitosan/propolis nano composite. It results in a considerable degree of dye absorption. The therapy produced high colour strength (K/S) values.. The treatment demonstrated that microwave heating is superior than ultrasonic heating. Time and energy can be effectively saved by using microwave. Additionally, compared to ultrasonic methods, it produces a better dye absorption and is more ecologically friendly. Natural dye need certain small-scale processes and little equipment, Natural dyes don't contaminate the environment . Given its quality, it appears to be a decent natural dying option. Wool fibers treated with chitosan/prpolis nano composite and dyed with madder natural dye had better antibacterial properties compared to *Escherichia coli* G^{-ve} against dangerous fungi including *Aspergillus niger* and *Staphylococcus aureus* G^{+ve} [22].

Author Contributions

Ali, N. F

1. Methodology.
2. Dye extraction ,purification and dyeing .
3. Investigation, Resources, Data.
4. Writing and Editing.

Abd- El salam, I. S.

1. Performing some experiments.
2. Methodology.
3. Resources, Data.
4. molecular identification of isolate & antimicrobial activity measurement

Declaration of competing interest

The authors declare that they have no conflict of interest.

Ethics approval and Consent to participate

No application

Data Availability

The identified fungal isolate was obtained from Chemistry of Natural and Microbial products Department, National Research Centre. Madder dye was extracted from madder roots.

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