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Development of extraction and application of alizarin from madder roots using supercritical carbon dioxide and its sustainable application on textile

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Abstract Low temperature, extraction pressure, critical carbon dioxide are the most commonly used supercritical condition. Super critical carbon dioxide was used to extract alizarin from madder roots (*Rubia tinctorum*) under various conditions, including temperature of 30–60 °C, pressure of 50–250 bar, extraction duration of 20–80 min, and flow rate of 5–9 mL/min. Under ideal circumstances, alizarin discovery in *R. tinctorum* extract (RE) of 1.34 g/kg roots with the concentration of 6.42 %. Wool textiles were dyed at various concentrations, pH levels, and times using microwave dyeing techniques. Low molecular weight chitosan was substituted for mordent at varying doses of 2–10 g/L. For both treated and untreated fibers, fastness characteristics of colored wool textiles were investigated. The dyeing characteristics and the fastness property were improved by the mordent. Technology was used to extract pigment of alizarin from *R. tinctorum* as a sustainable substitute for traditional extraction techniques. The impact of chemical, natural mordents, and extract concentration on the coloring characteristics of dyed wool textiles were assessed. Additionally, the antibacterial qualities of the dyed wool fabrics against bacteria and yeast including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Candida albicans* were assessed.

Keywords: Supercritical carbon dioxide**,** Alizarin**,** Wool, Dyeing, Antimicrobial activity

Introduction

Since the start of the twenty-first century, the contaminated ecological systems and reduction natural resources have caused a considerable deal of distress in the world (Wang *et al*., 2024). The balancing environmental protection for sustainability of textile dyeing and finishing industry is severely hampered by environmental issues such as limited water supplies, substantial unwanted water exposurefrom buildings involving with wastewater treatment (Broadbent *et al*., 2025; de Oliveira, 2024; Banchero, 2020). They need 8–18 MJ of energy, a substantial water volume over 100–150 liters/kg of fiber), and traditional

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dyeing or finishing techniques play a significant role in this issue (Pascual and Subra-Paternault, 2015; Abou Elmaaty *et al*., 2022).

A super critical CO_2 cylinder are used a chiller of a model Julabo FL601, semi-preparative CO_2 pump model JASCO PU-4386, interface box model LC-NeII/ADC, pressure regulator model JASCO BP-4340, cosolvent pump model JASCO PU-4180, temperature and speed controller model EYELARCX-1000 H, heater controller model HC-2068–01, 50 mL high-pressure stainless steel vessel and extract collection vial model HE-434001-200.

The highest flow rate of circulation pump revealed 10 mL/40 min. Supercritical CO_2 is the most widely performed in supercritical fluid extraction due to low temperature and pressure. Alizarin was extracted from madder roots (*R. tinctorum*) by using supercritical CO_2 under the conditions of co-solvent ratio at 0–50%, temperature of 45–95 °C, pressure at 150–250 bar, extraction time of 15–120 min, and flow rate of 5–9 mL/min. With this, Staphylococcus *aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Stapylococcus epidermidis*, and *Candida albicans* were treated to the dyed textiles resulted to be strong antibacterial activities. The color characteristics were enhanced by a higher RE concentration, the use of mordants, and specifically tannic acid.

Natural dyes are selected as sustainable sources of colorants because synthetic dyes are harmful and can trigger allergic reactions (Abd-Elsalam and Ali., 2024) In both natural and synthetic textiles, certain natural dyes offer color performance on par with some highly regarded synthetic dyes. (Abou *et al*., 2022; Yusuf 2019; Yusuf *et al*., 2017; Yusuf *et al*. 2013; AlAshkar and Hassabo, 2021). The primary ingredients of natural dyes are found to be anthocyanin, anthraquinone, flavonoid dyes, and polyphenol which showed a stable red hue. *R. tinctorum* belongs to Rubiaceae is the perennial herbaceous plant which is one of the oldest natural dyes to produce anthraquinones. Since 1500 BC, it has been commonly grown in Egypt, Iran, and Central Asia. It is considered as the most popular since it is found to be a natural source of gray, orange, purple, pink, and red as well as the most valued black and brunette hues with great color depth. CO_2 is frequently utilized as a solvent and to be an alternative to other solvents, it is cheap, non-toxic, and non-flammable, solvent which is easily accessible. Because of high volatility, then it rapidly extracted which can be preserved the properties of bioactive substances. Using the traditional dying method for the wool textiles showed the colored by using *R. tinctorum* root extract. This study is assessed how the concentration of extract and both natural and chemical mordant affected the dyed wool fabrics' coloring qualities. Additionally, the bactericidal qualities of RE and colored wool textiles were assessed which the aim to use in the manufacture for ecofriendly handloom carpets.

Figure 1. Chemical structure of the colouring matter (A), powder of dye (B) and dye roots (C) (Ali and El- Khatib, 2016)

Materials and method

Fibers

Wool fibers were supplied by El Mahalla company in Egypt. The supercritical fluid extraction was used the solvent of CO_2 99.6% which supported by NETCO Industrial Company, Cairo, Egypt which the used co-solvent as gradient-grade purity methanol of 99.8 %. Alizarin red was elucidated by high-performance liquid chromatography (HPLC) for separating using acetonitrile of 99.8%, and ammonium acetate. The conventional dyeing process was used aluminum sulphate (Alum) as a chemical mordant, and tannic acid was used as bio mordent.

R. tinctorum roots

R. tinctorum roots were collected from Damietta University's Faculty of Agriculture in Egypt. Plant roots were collected in accordance with applicable institutional, national, and international rules and regulations. They were properly cleaned with distilled water to get rid of contaminants, and then they were allowed to dry at room temperature. The dried roots were ground in a laboratory-scale mill (M 20; IKA, Staufen, Germany).

Dyestuff madder dye

The coloring substance used as a natural coloring matter was extracted from Madder as seen in Figure 1.

Extraction of natural color matter

R. tinctorum roots were collected at Damietta University's Faculty of Agriculture in Egypt. The dried roots were pulverized in a lab-scale mill (M 20; IKA, Staufen, Germany). Super critical CO_2 ($scCO_2$) was dissolved the target components by penetrating the plant matrix. The valve was adjusted to cool condiotion, liquefied scCO2 was flowed through the extraction vessel as dynamic circumstances which followed static time. In the dynamic mode of extracted components were transferred from the extraction vessel into the collecting system, and the plant matrix was applied with CO_2. The temperature was lowered to room temperature, and the pressure was lowered to ambient levels during the extraction period. $ScCO_2$ was lost solubility and changed to be gas phase as a result of breaking down. All extracts were gathered in collection vials and kept in a freezer at -20 °C.

Pre-treatment with chitosan low molecular weight

The solution of low molecular weight chitosan was prepared by dissolving (2.0 g/l) of chitosan into distilled water containing acetic acid (4g/l) and (2.0 g/l) of chitosan in distilled water containing citric acid (4g/l) according to the method of Yusuf *et al*, 2013 and Yusuf *et al*., 2017). The wool fibers were passed through microwave for five minutes after being submerged in this solution at a liquor ratio of 20:1, then the fibers were compressed and air dried.

Tannic acid pretreatment

The wool fibers were submerged in a 20:1 of liquor to tannic acid solution at concentration of 5%. The samples were done for five-minute in microwave treatment. Then, the fibers were pressed and allowed to air dry. (El-Khatib and Ali, 2016).

Mordanting with alum

The madder root was successfully adhered to fibers, a metal mordant is needed. Alum is the traditional mordant to use with madder as the well-known color in Turkish Red was created by combining alum, calcium, and madder. To ensure that the mordant was penetrated to fibers uniformly and deeply, tried to soak in water for a few hours prior to mordanting, The final color was rich, when the fibers were allowed to steep in the cooled mordant solution for two or three nights. When the fibers were dried and weighed, the samples were submerged in

a bath that contained both the dye solution and the mordant to perform simultaneous mordanting. The dyeing temperature was maintained for thirty minutes in 70 to 80 degrees Celsius (Ali and El-Khatib, 2016).

The process of dying

Wool fabric was dyed in microwave heating at pH 3-7 for varying durations of 1-5 minutes in a dye bath which contained different concentrations of 20 to 80 g/l of madder dye with a liquor ratio of 1:100. After being rinsed with warm and cold water, the dyed samples were placed in a bath with non-ionic detergent 5g/l at 50ºC for 30 minutes, then rinsed and placed to air dry at room temperature.

Alizarin measurement using high-performance liquid chromatography

High-performance liquid chromatography (JASCO, Tokyo, Japan) was used to perform for the chromatographic measurements with CO_2 pump model PU-4380, interface box model LC-NeII/ADC, UV/V is a detector (model UV-4070), SFC autosampler model AS-4350, RHPLC pump model PU-4180), column oven model CO-4060, and back-pressure regulator model BP-4340.The microsorb column-MV 100-5 C18 250 × 4.6 mm (Agilent Technologies, Santa Clara, USA), and JASCO software were used for data analysis.

Five standard solutions of alizarin at varying concentrations of 25, 50, 100, 150, and 200 µg/mL were done and dissolved in a 50:50 volume ratio of methanol and distilled water to quantify the amount of alizarin. The peak areas of alizarin were plotted to create calibration graphs. The mobile phase was a 60:40 (v/v) combination of acetonitrile and ammonium acetate (20 mM). Prior to use, the mobile phase was produced by filtered through a 0.45µm microsyringe filter and degassed for 20 minutes using an ultrasonicator (model S30H, Elmasonic, Singen, Germany). Twenty microliters were injected in triplicates. The elution program was run at 30°C for 15 minutes at absorbance of 254 nm with 1 mL/43min was chosen as the flow rate for detection.

Assessment of the fastness and color qualities

Color intensity (K/S values) and color coordinates were used as CIELab (lightness [L*], redness-greenness value [a*], yellowness-blueness value [b*], chroma [C*], and hue [h]) which measured by spectrophotometer (Konica Minolta, CM-3600, Tokyo, Japan). Each sample was analyzed at three locations in the left, middle, and right following the instrument's calibration. Kubelka-

Munk equation weas used for the color strength (K/S) values in the visible part of the spectrum of 400–700 nm which calculated as the link between reflectance and absorbance is described by this equation. The square root of the squares of the associated L*, a*, and b* differences was determined the color difference between any dyed and blank sample.

where L* is the lightness from black (0) to white (100), a* is the red (+)/green (-) ratio, b* is the yellow (+)/blue (-) ratio, and ΔE is the difference between the blank and dyed wool materials. The levels of dyed wool fibers using UV/V spectrophotometer (Alpha-1860, Noble, IN, USA) to measure the spectrum of alizarin in the dye bath both before and after the dyeing procedure. The color fastness of dyed wool samples was evaluated by ISO-standard procedures. The specific tests were used AATCC Test Method 61-1996 for washing fastness (AATCC Test Method 61 (2A)-1996), for durability testing (AATCC Test Method 16-2004) in light fastness (AATCC Test Method 8-2001) and to dry and wet rubbing fastness. Grayscale values are ranged from 1 (the worst) to 5 (the best) that evaluated for color changes and stains.

Antibacterial activity

Peptone 5.0 g, yeast extract 1.5 g, beef extract 1.5 g, NaCl 5.0 g, and agar 20 g at pH 7.5 was made as nutrient agar medium in 1L), then autoclaved at 121 °C for 20 minutes. Gram negative bacteria and gram-positive bacteria and fungi were used as test organisms. They were incubated overnight at 37 °C and 120 rpm in 2 mL of nutritional broth. The agar plates were seeded, wool samples were placed on the top of the seeded medium under sterile conditions, incubated overnight at 37 °C, then measured the zones of inhibition. The untreated wool samples were tested as control (Dhanalakshmi *et al*., 2013).

Results

Effect of dye amount

The extractability of Madder dye at various concentrations of 20 to 80 g/l) was noted. The concentration of the dye increased, after heating in microwave at 5 minutes. The color strength (K/S) of the dyed wool fibers increased after heating for one hour at the boiling temperature (Figure 2). Treatment of 40 g/l showed the best concentration of extraction after heating with microwave. The values of color strength of wool fibers confirmed that heating with microwave was more effective method than conventional heating.

Effect of extraction time

The extraction of madder dye was performed at concentration of 40g/l using microwave heating which done in 1-6 minutes, and conventional heating of 2 hours at the boiling condition. Result showed that the dye extraction by heating in microwave resulted to higher madder dye than traditional heating which maximum values revealed at 4 to 60 minutes respectively (Figure 3&4). The heating in microwave confirmed a uniform pattern of materials.

Effect of pH level of dye bath

Results revealed that pH values of 2 to 11 in the dye bath were affected the dye ability of wool fibers after using both dyeing techniques which showed by K/S value. Microwave irradiation and traditional heating were enhanced the dye characteristic ability at pH 3. It is proved that pH level in dye bath affected dye molecules and wool fibers. It explained that the madder dye which contains terminal hydroxyl groups interacted ionic in the terminal amino groups of wool fibers in acid condition through ionic exchange reaction. The appearance of weak hydroxyl anion of dye explained that acid condition was took place due to higher affinity, and anion of dye may possible be complex in nature to be bound to the fiber. It proved that the ionic interaction increased in the wool fibers dye (Figure 5).

***Effect of concentration of dye in dyeing* processes**

The dyed wool fibers were performed using natural dye extraction with madder at various concentrations of 20 to 80 g/l of a liquor ratio1:100. Wool fibers were dyed using microwave heating for 5minutes and conventional heating at boiling condition for one hour were compared.

Results revealed that K/S value in conventional method was increased when concentration of the dye increased to 60g/l. The heating in microwave showed the K/S value increased at 40g/l of the dye which gave more effective than the conventional dying method.

Effect of dyeing time

The effect of dyeing time illustrated the effect of dyeing time on the color strength as seen in Figures 7 and 8. The color strength was increased by increasing the dyeing time both methods of in microwave and traditional heating. As result, microwave heating revealed better than conventional method of dyeing

time when increased to 5 minutes, while traditional heating was 50 minutes. The heating in microwave showed that dyed fiber increased. The traditional heating method showed the dye was unevenly fibers which concentrated in a few parts of the outer areas. The heating of microwave showed better dye penetration which was higher and uniform of the dyestuff in fiber surface to make possible for diffusion to fiber interior.

Figure 2. Samples of wool fiber dyed with alizarin dye at different concentrations using chitosan low molecular weight

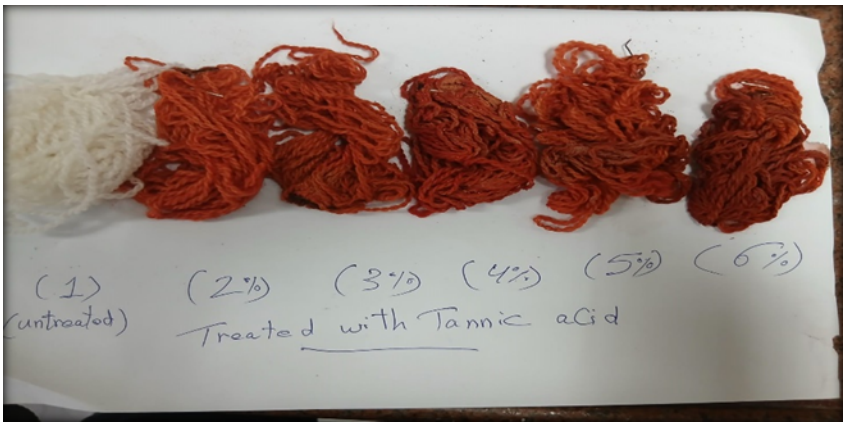

Figure 3. Samples of wool fiber dyed with alizarin dye at different concentrations using Tannic acid

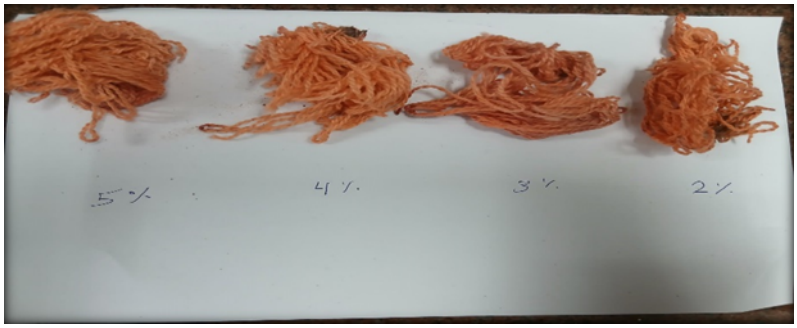

Figure 4. Samples of wool fiber dyed with alizarin dye at different concentrations using Alum

Effect of temperature on alizarin extraction efficiency

Result confirmed that the efficiency of extraction is affected by temperature. Aalizarin extraction was determined at temperatures of 45 and 95 °C in static conditions. It revealed that alizarin decreased when temperature increased to 65 °C. It explained that temperature affected both extraction methods. It reduced the viscosity of solvent and increased compound diffusivity but can be considered to improve the efficiency extraction. It is confirmed that $scCO_2$ density decreased at high temperatures which resulted from thermal degradation of matrix components and excessive extraction. The degradation of thermolabile compounds can be minimized by supercritical fluid extraction temperature after adjusted to 35 and 60 °C. The most suitable temperature for extraction was 65 °C for alizarin production from roots of R. *tinctorum* (Figure 5) with aadjusted theoptimum temperature to 65 °C, flow rate, optimization of pressure, and extraction time.

Effect of pressure on the efficiency of alizarin extraction

The extraction efficiency of alizarin was affected by presser when using the SFE technique. The roots of *R. tinctorum* were performed extraction in different pressure levels of 150, 175, 200, 225, and 250 bar. The co-solvent ratio, extraction time, flow rate and temperature were set up to be constant at 90 CO_2:10 Me, at 65°C, for 60 minutes with 9 mL/min, respectively. Alizarin production was increased when pressure increased in 150 bar pressure. Alizarin production was used at 1.23 kg of roots increased production to 1.34 g/kg at 250 bar pressure. As comparison of two methods, alizarin content in the RE increased in pressure from 150 bar to 250 bar, and the alizarin content in the RE increased from 5.69 to 6.18% (Figure 5). The increasing pressure resulted to increase thge

density and diffusivity of CO_2. The pressure was increased; the fluid can be interacted with the matrix to improve the extraction efficiency and solubility of alizarin. It proved that the optimum pressure level of 250 bar was maximized for alizarin extraction efficiency.

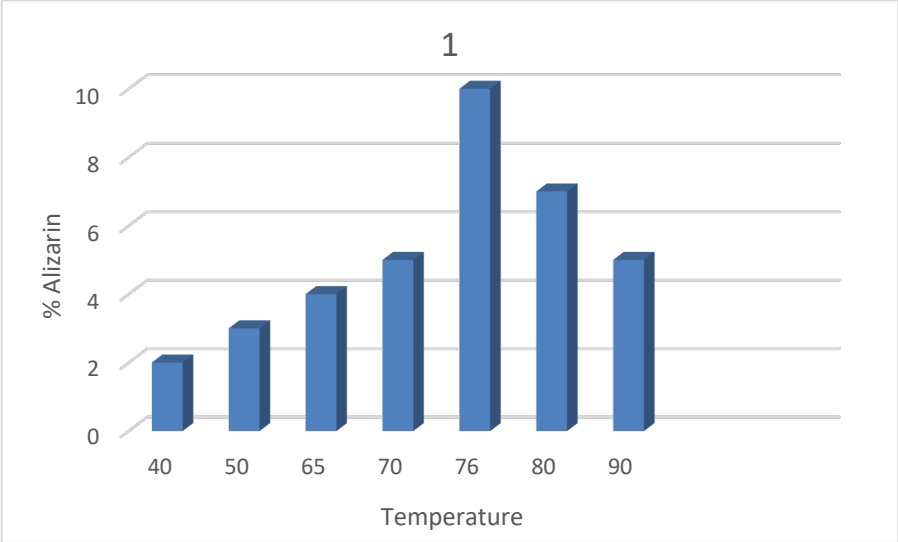

Figure 5. Effect of temperature on alizarin extraction efficienc

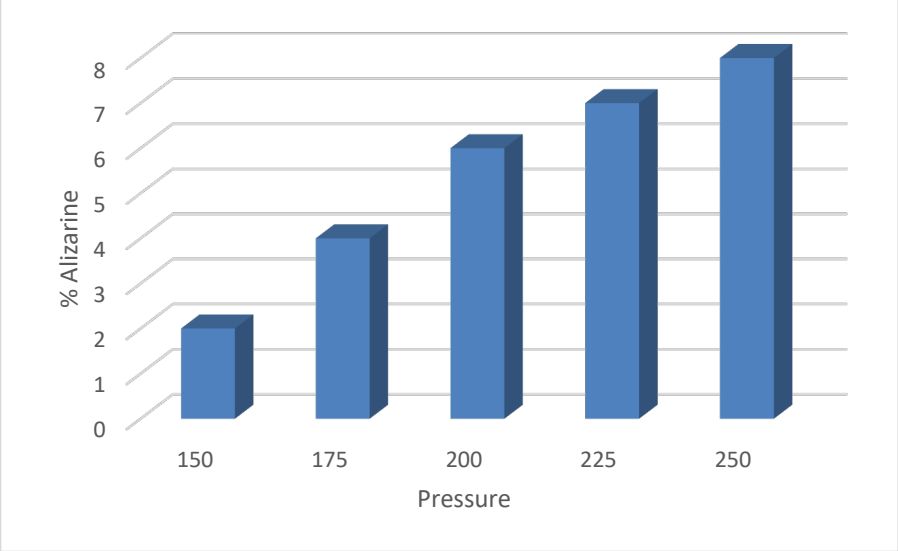

Figure 6. Effect of pressure on the efficiency of alizarin extraction

Alizarin extraction efficiency as a function of extraction time

The ideal time for alizarin recovery in SFE can be found by examining the impact of extraction time on alizarin extraction efficiency. If the extraction period is too short, an incomplete extraction may result. On the other hand, if the extraction process took too long, it wasted solvent and time, which increased the cost of extraction. The roots of *R. tinctorum* were extracted at various time of 15, 30, 45, 60, 90, and 120 minutes at a steady flow rate of 9 mL/min while all other parameters were adjusted to their levels. Alizarin production increased from 0.66 to 1.73 g/kg roots as extraction time increased. The pace of rise slowed after 45 minutes. The optimization flow rate was 45 minutes. Alizarin concentration in the RE was maximized for extraction efficiency to enhance the longer time between scCO2. The value of alizarin percentage in the RE decreased with time of extraction (Figure 7).

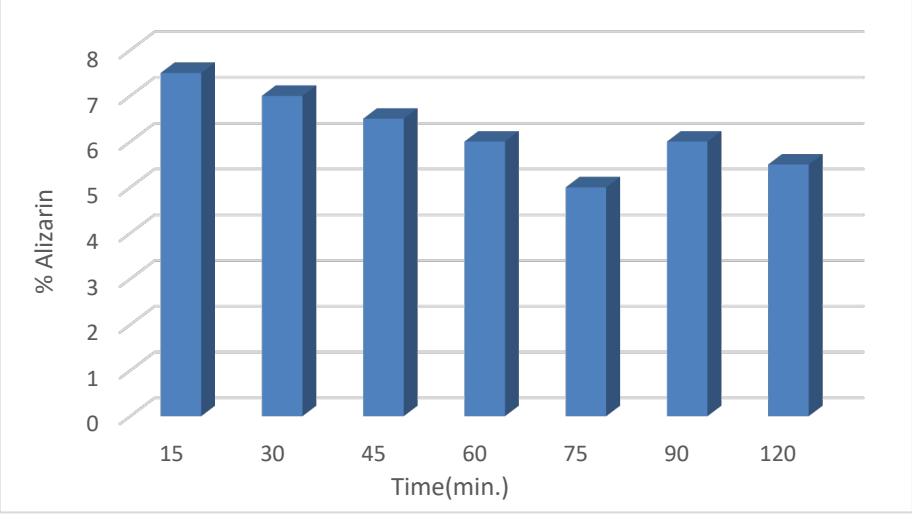

Figure 7. Effect of Time on the efficiency of alizarin extraction

Table 1. Colour data of wool fibres pretreated with tannic acid and dyed with madder at different concentrations

Conc. of Tannic acid	K/S	L*	a*	b*	C*	ΔH	Δ E
0.5	6.3	76.74	18.5	9	22	24.5	41
1.0	10.42	46.71	32	14.3	34.5	25.2	53.2
1.5	15.31	46.66	34.6	15.5	36	25.5	54.83
2.0	16.5	37.72	36.5	16	37	26	57
Untreated	7.5	41.39	35.7	21.2	40.5	29.5	30

Table 2. Colour data of wool fibres pretreated with alum and dyed with madder at different concentrations

Conc. of Alum	K/S	L*	a*	b*	C*	ΔH	ΔE
0.5	2.52	76.74	44	4.7	44	6.9	57.8
1	4.3	44.5	44.54	11.65	45	13	51.3
1.5	22.87	59.8	57	20	60.2	19.5	71.6
2	7.6	42.3	52.5	14	54.5	15	64.3
2.5	5	49.5	50	15	52.7	16	59

Impact of the pretreatment on microwave dyeing

Wool fiber dyeing was improved by the pretreatment with chitosan, tannic acid, and alum. It formed chemical connections with the functional groups found in the side chains of the constituent amino acids or with the terminal -NH2 or -COOH groups of the polypeptide chain. microwave-dyed wool fibers. As seen in Figures 8, 9, and 10, the treated samples had greater K/S values than the untreated samples at different concentrations, pH levels, and times. As a mordant, tannic acid creates chemical connections of dye molecules and fibers and changing color of the produced dye.

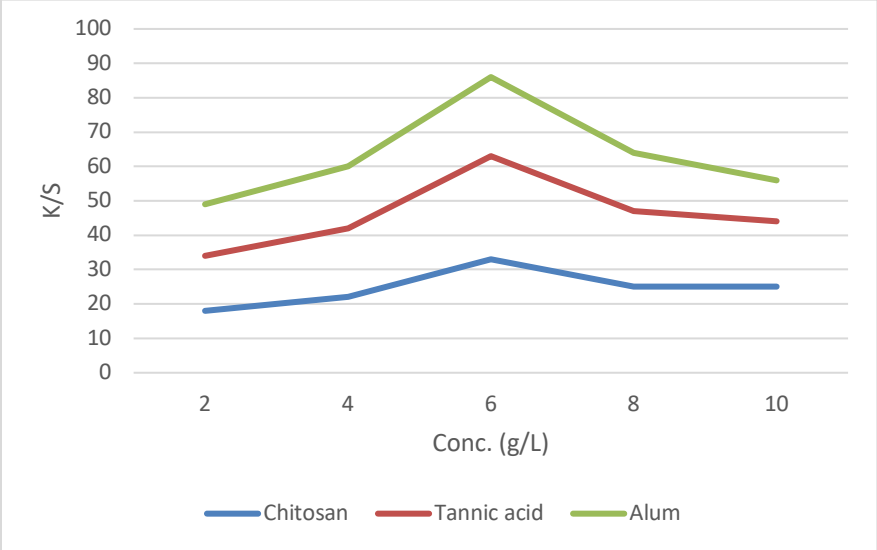

Figure 8. Effect of concentration of chitosan, tannic acid and alum

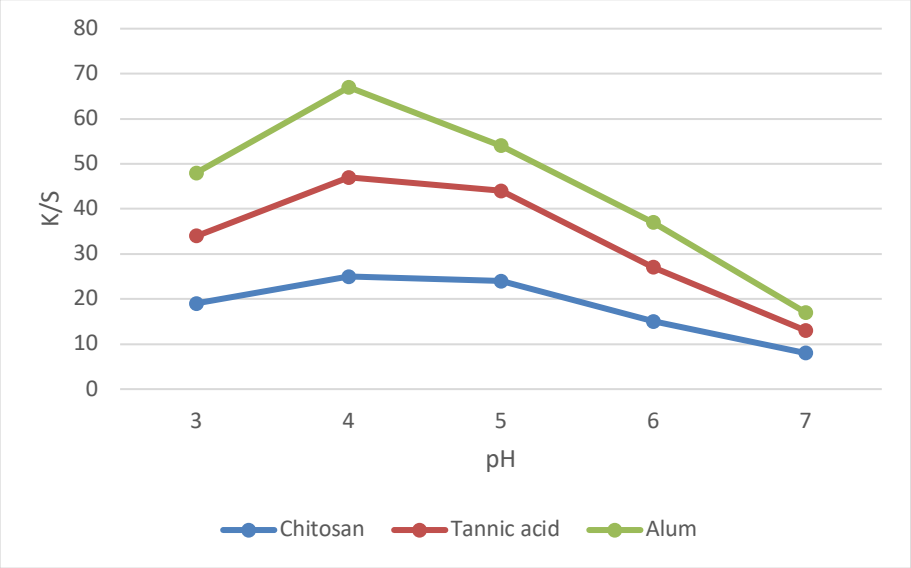

Figure 9. Effect of pH of chitosan, tannic acid and Alum

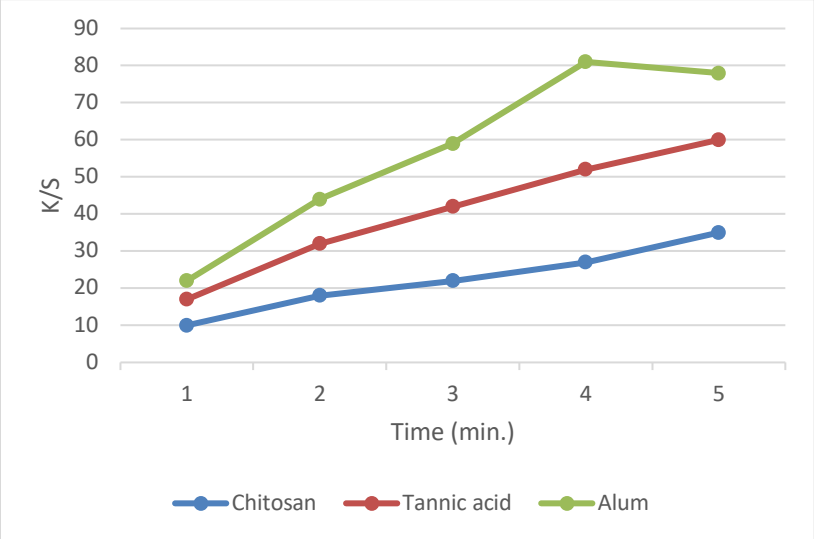

Figure 10. Effect of dyeing time using chitosan, tannic acid and alum

Antibacterial activity

The effect of tannic acid at different concentrations of 2,3,4,5 % expressed actively against different gram-negative bacteria (*E. coli* and *P. aerogenosa*) and gram-positive bacteria (*S. aureus*) as well as yeast (*C. albicans*) as noticed in Table 1. It showed that tannic acid at 5 % was the maximum inhibition against either gram negative and gram-positive bacteria (2.45,0.89 and 0.95 cm) respectively. The alum at different concentrations of 2,3,4,5 % was actively against different gram-negative bacteria (*E. coli* and *P. aerogenosa*) and gram-positive bacteria (*S. aureus*), and yeast (*C. albicans*) (Table 2). It revealed that tannic acid at 5 % was the maximum inhibition against gram negative and gram-positive bacteria.

Table 3. Effect of Tannic acid and the tested concentrations

Tannic acid	**Inhibitionzone (cm)**			
Conc. (%)	***E. coli***	***S. aureus***	***P. aerogenosa***	***C. albicans***
control	-	-	-	-
2	0.58	-	-	-
3	1.89	1.20	0.68	-
4	2.45	0.95	0.89	1.14
5	2.1	0.55	1.00	1
6	2,3	0.51	1.00	1

Table 4. Effect of different concentration of Alum

	Inhibition zone (cm)			
	E. coli	***S. aureus***	***P. aerogenosa***	***C. albicans***
control	-	-	-	-
2	0.34	-	0.34	-
3	0.92	0.45	0.33	0.50
4	0.89	0.55	0.35	0.57
5	1.45	0.34	0.45	0.59

The minimum inhibitory (MIC) showed that the used dye had positively affected on *E coli* and *S. aureus* at concentration of 150 ug/ml / while the tested yeast (*C. albicans*) showed a defense against this concentration, it can stand till concentration of 200 ug/ml. These results revealed that the used dye was strongly

affected gram negative and gram-positive bacteria with slightly activity against yeast (Figure11).

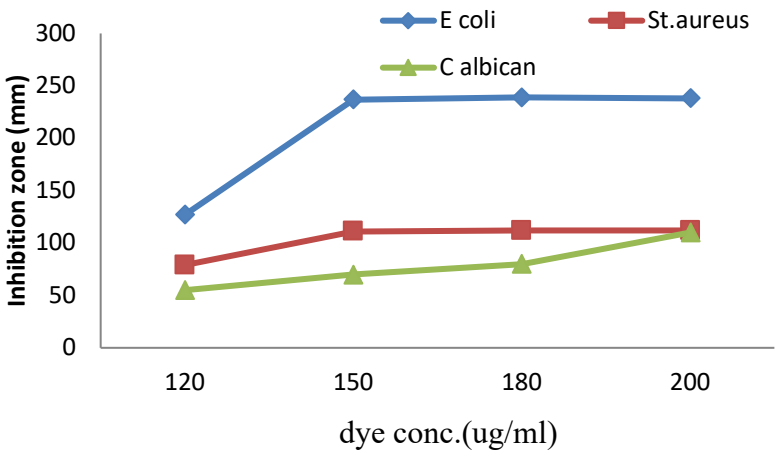

Figure 11. Minmum inhbitory con. (MIC). of the used dye against selected bacteria and yeast

Fastness characteristics of microwave-dyed wool fabric

It showed that the wool fabric samples were maintained the color after dyeing and their interactions with fibers (Table 5). The fastness properties of the dyed materials demonstrated that the colored samples was a good fastness. The fastness of microwave radiation showed a heating source in terms of rubbing, washing, sweating, and light fastness by a grey scale. The results revealed that wool fiber was more resistant for washing, and color intensity in microwave radiation method which increased in dye penetration.

Table 5. Fastness properties of the dyed wool using Alizarine

Mordent	Rubbing fastness		Washing fastness			prespiration fastness						Fastness to light
						Alkaline			Acidic			
	Dry	Wet	Alt	Sc	Sw	Alt	Sc	Sw	Alt	Sc	Sw	
Chitosan	4	4	3	5	-5	4	4	4	3-4	4	5	6
Tannic acid	5	3-4	5	-5	4	4-5	4	4-5	4	4	4-5	7
Alum	5	4-5	4	4	4-5	4	4-5	4	4	45	5	7

Alt: alteration Sc: staining on cotton, Sw: staining on wool

Discussion

The bactericidal qualities of dyed textiles were examined. The effectiveness of dyed fabric against *P. aeruginosa* was higher than that of other bacterial strains. Alizarin molecules released which altered the bacterial cell wall and prevented bacterial development, is thought to be responsible for the antibacterial effect. (Ali and Abd-Elsalam, 2024). The effect of tannic acid at 5 % showed the maximum inhibition against the tested microorganism. (Dhanalakshmi *et al*., 2013). The minimum inhibitory (MIC) showed that the used dye had positively affected on *E coli* and *S. aureus* at concentration of 150 ug/ml / while the tested yeast (*C. albicans*) showed a defense against this concentration. The environment is affected organic solvents which decreased by using SFE and supercritical technologies to extract natural colorants. Temperature has a significant impact on extraction efficiency. Alizarin recovery was measured at various temperatures in this investigation. Extraction also affected by pressure and extraction time (Broadbent *et al*., 2025). Microwaves heating is more effective for dyeing with Madder Wool fibers absorb more dye as a result. Compared to traditional heating, the improved effect was almost 50% greater. Additional characteristics of microwaves are their increased dye uptake, cost-effectiveness, and environmental friendliness. and good color difference as L*, a*, and b* as compared to conventional techniques. The pretreatment by chitosan low molecular weight, tannic acid and Alum leads to larger K'S values for wool fibers than the untreated dyed wool fibers (Ali and El- Khatib, 2016). Wool fibers dyed with madder natural dye exhibited the best fastness properties in microwave heating method

Conflicts of interest

The authors declare no conflict of interest.

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