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Using Ultrasound and Cellulase Enzyme as a Clean and High-Efficiency Extraction Process for Rubia tinctorum L

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Abstract

This study introduces an alternative cleaner method to extract dyes existing in madder using ultrasound and cellulase enzyme. Also, in conjunction with the bio-mordants, wool dyeing was investigated as a cheap and environmentally benign alternative for inorganic mordants. In this respect, the optimum extraction condition achieved from the central composite design was 35 mL/L enzyme, 45 min, 30%(v/v) alcohol in buffer solution (pH 7). The results indicated that dye extraction yield was 64% in this method, while it was 55% for the three-step ultrasound-assisted method and 48% for the five-step classical method. Overall, a fast and efficient process has been proposed in this study for extracting dye from madder. The extracted dye was applied to the dye of mordanted wool yarns with walnut hulls, pomegranate peels, myrtus communis leaves, and amygdalus scoparia root as bio-mordants. Results revealed the extracted dye can dye wool

yarns with good build-up and acceptable color fastness properties.

Keywords**:** Bio-mordant; Eco-friendly method; Enzymatic extraction; Madder; Ultrasound; Wool dyeing.

1. Introduction

It is difficult to overestimate the importance of natural dyes for the dyeing of textiles in eco-friendly processes as well as its popularity globally. The chemical structures of dyes, their contents, and environmental aspects are important in producing sustainable and cost-effective dyes from natural sources with high exhaustion and acceptable color fastness properties [1, 2]. Madder is an industrial plant and a member of a large botanical family, known as the Rubiacea family including over 6500 species, and 450 genera, most of which are large woody plants [3]. This plant is one of the most traditional colorants for creating red hues and diverse shades with a high color depth on textiles [4]. The classical method for madder extraction is more efficient through extraction repetition by fresh solvents in any step. However, the classical extraction methods have some serious drawbacks in the extraction efficiency and processing time. To extract dyes from plants in a clean and environmentally acceptable manner, modern techniques including microwave, gamma, and ultrasound have been used, either alone or in conjunction with enzymes [5-8].

Ultrasonic extraction of dyes has attracted much attention for higher mass transfer and chemical bond breakdown associated with cavitation phenomena and considerable diffusion acceleration [9]. Ultrasound leads to a higher extraction yield while reducing

the processing time and lower chemical and energy consumption in comparison with conventional techniques [10, 11]. The combination of ultrasound and enzyme led to higher efficiency in the extraction process. Derksen et al. explained different methods using acid, base, or enzymes to extract madder roots via the hydrolysis extraction method [12]. There is some research about the application of enzymes and ultrasound together in the extraction of natural dyes. However, there is no report on the use of the enzyme immediately after sonication. In most cases, the enzyme is first added to the solution followed by the sonication. As the researchers pointed out, however, it can destroy the enzyme and reduce its effectiveness. In some cases, sonication is first performed, followed by the filtration and addition of the enzyme to the cell debris for hydrolysis and release of dye molecules from sugar moiety. This method involves several steps, with high solvent consumption [13]. We suggested a new, quick, effective, and hygienic technique for the one-step extraction of madder dye that combines sonication and enzyme (UE) to get over these problems. This approach completes the extraction process right after sonication by adding the enzyme to the solution and stirring it concurrently. Therefore, this method needs less time, energy, and solvent. To attain optimum extraction conditions, the response surface methodology (RSM) was used and the influencing factors, such as enzyme concentration, time, solvent concentration, and pH were investigated. Walnut hulls, pomegranate peels, myrtus communis leaves, sumac, myrobalan, and amygdalus scoparia root are tannin-rich sources that can be used as bio-mordants in wool dyeing with extracted madder dyes [14, 15]. Therefore, we studied the effect of various concentrations of the bio-mordants and madder dye extracts on the dyeing properties of all dyed samples.

2. Experimental

2.1. Materials and instruments

The wool yarn with 200 Tex/four folds (Azarbarf, Iran) was used. Cellulase enzyme was obtained by Novozymes Co. Labolene as a non-ionic and phosphate-free detergent prepared by KK International Company (India). Rubia tinctorum L (Madder) and biomordants were provided from Yazd province (Iran). The plant materials were dried to the minimum surface moisture level at ambient temperature for 2 weeks and then milled by a laboratory mill apparatus (Volta, 2500 M, Iranpar). Other chemicals were purchased from Merck, Germany. The ultrasonic treatment was performed in a Hielscher UP400S (Germany, 24 kHz, and 400W). A laboratory HT dyeing machine (Rapid, India) was used for wool dyeing. The absorption spectra of the dye solutions were measured by a double-beam spectrophotometer (CECIL 9200 series). The reflectance properties of the dyed samples were measured using Color-Eye XTH Spectrophotometer, X-Rite Inc, USA, under D65 illumination, 10° standard observer.

2.2. Methods

2.2.1. Cellulase activity assay by DNS method

An aqueous organic solvent containing water/ethanol was used to extract dyes present in the madder. Therefore, cellulase activity was investigated using DNS (3,5-dinitrosalicylic acid) at extraction and reference conditions [16]. A solution containing 1% (w/v) carboxymethyl cellulose (CMC) was prepared in 100 mM sodium dihydrogen phosphate buffer (pH 7.0). Several concentrations of substrate were added to 20 µl of enzyme solution, after which the volume of solution was diluted to the final volume of 500 µl by

adding buffer (100 mM sodium dihydrogen phosphate), alcohol/buffer solution (1:1), After incubation at 45 °C for 15 min, DNS (500 µl) was added to the solution. The mixture was immersed in boiling water for 10 min. Then the absorbance of the solution was recorded at 540 nm. V_{max} and K_m parameters for the cellulase enzyme were determined.

2.2.2. Classical and ultrasonic extraction of madder dye

Madder roots (5 g) were added to ethanol/water (1:1) solution (250 ml) at pH 7, at 50°C under stirring at 250 rpm. The samples were filtered and centrifuged (400 rpm, 10 min), and then the absorbance of dye-extracted solutions was measured [17]. This method was repeated five times on the remaining powder until all extracted dyes from the madder and the absorption intensity reached the maximum value of 0.1. In the case of dye extraction by ultrasonic method, the crude powdered madder (1 g) was added to ethanol/water (1:1) solution (50 mL). The ultrasonic probe (100 W) with pulse mode operation (40 s on/60 s off) was used to prevent the bath temperature rise and dye degradation (4, 7, and 10 pulses). During sonication, the temperature and pH were adjusted to 50°C and 7, respectively, as the control experiment. The obtained dispersion was shaken at 25, 40, 50, and 60˚C to determine the optimum extraction temperature. The samples were then filtered and centrifuged (4000 rpm, 10 min), after which a spectrophotometer was applied to measure the dye absorption. Dye absorbance was calculated according to Eq. 1. Finally, the extraction efficiency of each sample was determined (control experiment).

Dye absorbance = Dilution coefficient × Solution absorbance (1)

2.2.3. One-step extraction of madder dye by using ultrasonic and cellulase enzyme

Thirty experiments were achieved using CCD design (Table 1). The influence of different variables such as enzyme concentration (10-80 ml/L), time (10-90 min), alcohol concentration (0-100 v/v) in alcohol/buffer solution, and pH value (5-9) were investigated on the response. 1 g of crude powdered madder was added to ethanol containing sodium dihydrogen phosphate buffer (0.1 M) at different percentages and specified pH based on statistical design. The ultrasonic probe was used with ultrasonic pulse mode (40 s on/60 s off) four times. The suspension was treated with enzyme solutions based on a statistical design. The extraction of dye was carried out by a shaker incubator at 50 °C with stirring (80 rpm) for selected times. The stirring rate was increased to 280 rpm for the predetermined stirring time of 15 min.

Table 1: CCD design for extraction madder dye.

Run	Factors				Response
	Enzyme (ml/L)	Time (min)	Alcohol %(v/v)	pH	Absorbance*
1	27.6	30	25	6	9.49
2	62.6	30	25	6	8.71
3	27.6	70	25	6	9.88
4	62.6	70	25	6	9.49
5	27.6	30	75	6	11.83
6	62.6	30	75	6	10.4
7	27.6	70	75	6	11.57
8	62.6	70	75	6	11.7
9	27.6	30	25	8	10.14
10	62.6	30	25	8	9.75
11	27.6	70	25	8	10.01
12	62.6	70	25	8	9.75
13	27.6	30	75	8	12.35

14	62.6	30	75	8	11.57
15	27.6	70	75	8	11.31
16	62.6	70	75	8	11.96
17	10.0	50	50	7	11.31
18	80.0	50	50	7	10.79
19	45.0	10	50	7	12.22
20	45.0	90	50	7	12.35
21	45.0	50	0	7	5.72
22	45.0	50	100	7	10.14
23	45.0	50	50	5	10.53
24	45.0	50	50	9	11.05
25	45.0	50	50	7	11.44
26	45.0	50	50	7	11.44
27	45.0	50	50	7	11.31
28	45.0	50	50	7	11.18
29	45.0	50	50	7	10.92
30	45.0	50	50	7	11.44
31	0	45	30	7	8.58

* Dye absorbance was calculated based on the dilution coefficient.

2.2.4. Extraction of bio-mordants

Each selected bio-mordant was kept in an ethanol/water 50:50 aqueous solution with liquid ratio L.R of 40:1, at boiling temperature for 1 h. The extracted solutions were filtered and dried at 50 °C and used for the pre-mordanting of wool yarn samples.

2.2.5. Mordanting and dyeing

The wool yarns were scoured with a non-ionic detergent (5 g/L, Labolene) at 50 °C for 30 min, and then pre-mordanted with extracted bio-mordants with the concentrations of 5, 10, 20, and 40% (L.R of 40:1). Mordanting was initiated at ambient temperature. The temperature was then increased to 100 °C in 30 min and kept at this temperature for 1 h,

followed by washing. The wool yarns (2 g) were dyed with a dyeing apparatus using acetic acid (pH 4-5) and the L.R of 40:1. Dyeing of yarns was performed using dye solutions with 10-60% (o.w.f) of madder, and 1, 5, 10, 15, 20, 25, and 30% ultrasound/enzyme-assisted extracted dye powder for pre-mordanted yarns with 8% aluminum sulfate. Also, the dyeing of mordanted yarns with 5, 10, 20, and 40% bio-mordants was performed using dye solutions containing 10, 20, and 40% extracted dye. Kubelka-Munk equation was used to calculate the color strength (K/S) of the dyed samples at λ_{max} for each sample [18]. The color fastness of samples was determined according to ISO 105 C06 C2S:2010, ISO 105 B02:2013, and ISO105-X12:2016 standards.

3. Results and Discussion

3.1. Effect of ultrasound

The efficiency of dye extraction from madder in classical and ultrasound-assisted extraction was 48 % and 64 %, respectively. Ultrasound-assisted dye extraction increased the extraction yield by about 16% in comparison with the classical extraction. Acoustic cavitation grows by ultrasound and eventually bursts into microbubbles. This increases the local temperature in less than a microsecond, releasing large amounts of short-lived hot spots, microjets, and shock waves near a solid surface [19]. Furthermore, ultrasound irradiation increases dissolution, mass transfer, as well as the surface area between the reactants and the solid in the surrounding liquid phase [20]. Ultrasound facilitates the extraction and transfer of dyes to the solvent.

3.2. Effect of cellulase enzyme on madder dye extraction

Using the Prism program, the V_{max} (the maximum reaction velocity) and K_m (the

substrate concentration at which half of the maximum velocity is achieved) parameters of the cellulase enzyme were determined as 0.098 $mM.min^{-1}$ and 100 µg, respectively. The specific activity of the enzyme was 10 $U.mg^{-1}$. An aqueous organic solvent containing a mixture of water/ethanol was used to extract madder dye. Therefore, cellulase activity was investigated in extraction and buffer media (Table 2). Results demonstrated that the cellulase enzyme was active in the extraction medium.

Table 2: Cellulase enzyme activity at different solvents.

Sample	Substrate(µL)	Buffer(µL)	Alcohol (µL)	Enzyme(µL)	Absorbance
1	200	250	250	50	0.94
2	200	500	-	50	1.85
3	200	-	500	50	1.45

It is recommended that the enzyme concentration can be reduced by 35 ml/L when using ultrasound. This leads to a high dye extraction efficiency of about 64% compared to ultrasound and classical extraction methods of 55 % and 48 % dye extraction efficiency, respectively. The improvement in extraction efficiency via the UE can be directly demonstrated by dye absorbance of about 11.28 in one step of extraction obtained for ultrasound 8.58 extraction in three steps, and classical extraction of 3.60 in five steps.

The solid-liquid leaching process is one of the main problems in dye extraction due to the strong linkages between dyes and the cell membranes of plants. Enzymes have large chemical structures and only interact with the outer surface of cellulosic compounds in crude natural dyes, where enzymatic extraction needs to be combined with improved methods such as ultrasound. Pre-sonication of crude natural dye improves enzyme activity in breaking the cell wall by increasing the enzyme accessibility to the interior of

the madder [21].

The enzymatic extraction involves transferring cellulase enzyme from an aqueous solution to a plant surface through affinity, where binding of cellulose molecules by bulk leads to the formation of an enzyme-substrate (E-S) complex. Catalysis of the hydrolytic reaction leads to the transferring of the extracted dyes to the solvent medium [22]. Breaking down various bonds in the madder with ultrasound irradiation leads to deeper enzymatic attacks and more effective hydrolytic reactions. Adsorption of cellulase enzyme on the cellulosic part of the madder is a solid/liquid process. The enzymatic process was carried out after ultrasound treatment. The results of dye absorbance at different enzyme concentrations are shown in Figure 1.

The ultrasound irradiation improves the transfer of cellulose molecules from the madder to the aqueous phase, facilitating the diffusion of cellulase enzyme onto the madder. Enzyme leads to the breakdown of glycoside bonds on madder and promotes dye extraction in the liquid medium, resulting in a high extraction efficiency of about 16% compared to that of the classical method. Most dyes are extracted by UE extraction (Figure 2).

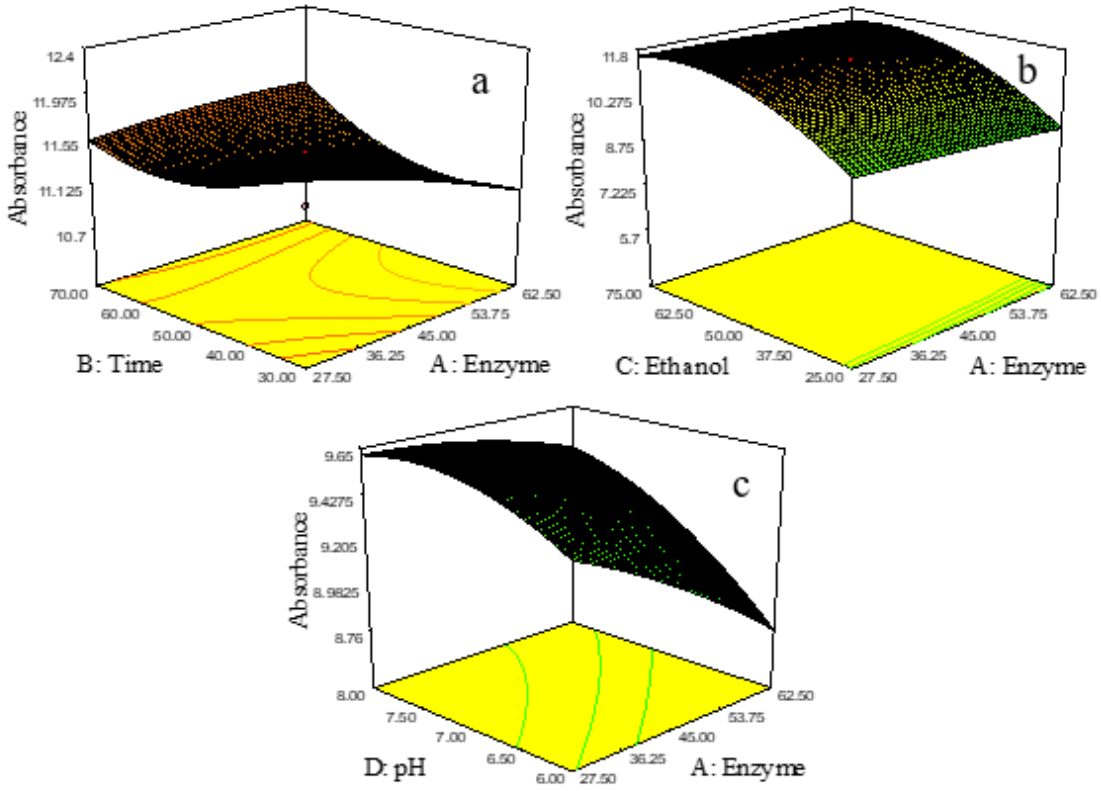

Figure 1: The surface response related to dye absorption in UE extraction madder dye; Interaction between (a) enzyme concentration and time, (b) enzyme concentration and alcohol percentage, (c) enzyme concentration and pH.

Figure 2: Remain powder of madder root in methods involving a) classical, b) ultrasound, c) UE extractions

3.3. Optimization of extraction process by RSM

The quadratic model was chosen as the most convenient model because it had the best-fitted data and lower total error relative to other models (Table 3). The correlation coefficient illustrates the satisfactoriness of the model to describe the system behavior in the range of operating factors. The closeness of this parameter to 1 indicates that the validity of the model is significant. R^2, R^2-adjusted and R^2-predicted values were 0.982, 0.965, and 0.92, respectively. The variation coefficient (CV %) of about 2.3% indicates the acceptable reliability and high accuracy of the experiments. In addition, the p-value was less than 0.0001 confirming that the selective model was acceptable.

Table 3: Sequential Model Sum of Squares

Source	***Sum of Squares***	***df***	***Mean Square***	***F Value***	***p-value Prob > F***	
Mean vs Total	3453.56	1	3453.56			
Linear vs Mean	26.47	4	6.62	6.52	0.0010	
2FI vs Linear	1.55	6	0.26	0.21	0.9704	
Quadratic vs 2FI	22.88	**4**	5.72	92.18	< 0.0001	**Suggested**
Cubic vs Quadratic	0.63	8	0.079	1.87	0.2130	Aliased
Residual	0.30	7	0.042			
Total	3505.40	30	116.85			

The plots of residuals versus fitted contents and the normal probability plots for madder dye extraction are presented in Figure 3a. The fairly straight lines formed by the points clearly show that the normality assumption for dye extraction is relatively reasonable. By plotting residuals as a function of fits it is possible to check the acceptability of the model's fit. In a reliable model, there should be no increasing residuals with increasing

fits, decreasing or increasing points, and predominance of negative or positive residuals. The plots shown in Figure 3b exhibit residuals with acceptable behavior for dye. So, it can be concluded that the practical model is adequate to illustrate the extraction of dye from madder by RSM.

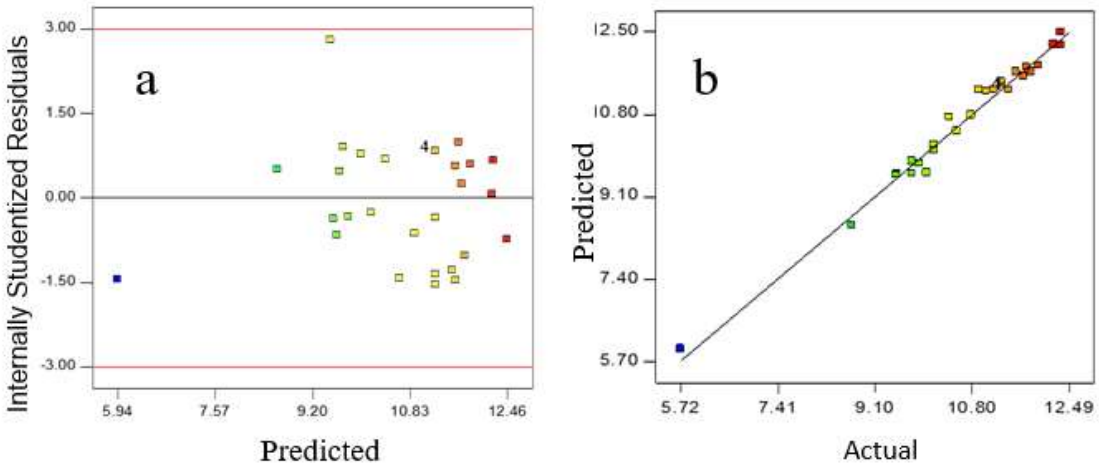

Figure 3: a) Residual-Predicted plot and b) Actual-Predicted plot.

An equation was obtained by Design-Expert software, representing optimum conditions for dye extraction (Equation 2).

Absorbance (a.u.) =11.31-0.18A+0.07B+1.01C+0.20D+0.22AB+0.024AC +0.11AD -0.041BC-0.19BD-0.024CD-0.047A^2+0.26B^2-0.83C^2-0.11D^2 (2)

Where A is enzyme concentration (ml. L^{-1}), B is extraction time (min), C is alcohol concentration (v/v) in alcohol/buffer solution, and D is pH. In this equation, parameters with p-values more than 0.05 were omitted since they have no remarkable effect on absorbance. Therefore, equation 1 was modified to equation 3.

Absorbance (a.u.) =11.31-0.18A+1.01C+0.20D+0.22AB-0.19BD+0.26B^2-0.83C^2-0.11D^2 (3)

It was found that the selected operational parameters such as enzyme concentration, time, pH, and solvent affect the efficiency of the extraction process, with the absorption intensity varying between 5.72 and 12.35 (Table 4). This amount was 8.35 for the extracted sample without enzyme addition

Table 4: ANOVA related to the absorption intensity of different conditions.

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Value*	*p-Value (Prob›F)*	
Model	50.91	14	3.64	58.59	‹0.0001	significant
A(Enzyme)	0.77	1	0.77	12.36	0.0031	
B(Time)	0.12	1	0.12	1.92	0.1864	
C(Ethanol)	24.62	1	24.62	396.77	‹0.0001	
D(pH)	0.96	1	0.96	15.53	0.0013	
AB	0.77	1	0.77	12.41	0.0031	
AC	9.506E-003	1	9.506E-003	0.15	0.7010	
AD	0.18	1	0.18	2.88	0.1105	
BC	0.026	1	0.026	0.43	0.5241	
BD	0.56	1	0.56	9.00	0.0090	
CD	9.506E-003	1	9.506E-003	0.15	0.7010	
A^2	0.062	1	0.062	0.99	0.3349	
B^2	1.87	1	1.87	30.19	‹0.0001	
C^2	18.78	1	18.78	302.56	‹0.0001	
D^2	0.35	1	0.35	5.17	0.0321	
Residual	0.93	15	0.062			
Lack of FIT	0.69	10	0.069	1.47	0.3521	not significant
Pure Error	0.24	5	0.047			
Core Total	51.84	29				

R-squared: 0.9820, Adjusted R-squared: 0.9653, CV%: 2.32.

The three-dimensional response surface of the polynomial is shown in Figure 1a-c. The enzyme concentration plays a key role in the extraction process via the UE method. Indeed, the enzyme concentration factor is very significant in the extraction process, where the extraction yield increases in the presence of cellulase enzyme. The impact of various concentrations of cellulase enzymes on the extraction process of madder dye is

shown in Figure 1. Increasing enzyme concentration up to 35 ml/L increased the dye absorption, resulting in better dye extraction. Higher concentrations led to a small decrease in dye extraction because of the saturation of the solution. Thus, 35 ml/L was selected as the optimum cellulase enzyme concentration.

The pH value plays a key role in enzyme activity since enzymes are active at their optimum pH [23]. Therefore, dye extraction was performed at different pH values (5-8) to investigate its effect on the absorbance, as presented in Figure 1. The dye absorption intensity from the extracted madder increased by increasing the pH values, with the highest yield (12.35) obtained when the pH was 7. The results indicated that the optimum pH of 7 was suitable for the enzymatic extraction of dye in the present experiment. Inactivation rates of cellulase enzymes and enzymatic hydrolysis rates of madder depend on the pH and processing temperatures. At pH 6 and 45°C, the glycosides of madder conversed in ultrapure water, and the changes disappeared in less than 45 min.

The dye absorption increased from 9.45 to 11.48 as the alcohol concentration in the alcohol/ buffer solution increased from 12.5 to 37.5 (Figure 1). Buffer is necessary to adjust and stabilize the desired pH during the enzyme assay. On the other hand, the extraction of anthraquinone compounds in madder roots can occur more easily in ethanol as a polar solvent. The results in Table 2 indicate that the cellulase enzyme was active in both aqueous and organic solvents as extraction media for madder dyes. Thus, an alcohol concentration of 30% was chosen as the optimum ratio.

The effect of solvent percentage and pH on dye absorbance is the same as that of enzyme concentration. The time had no meaningful effect on the extraction yield. However, considering interaction effects, the time was significant in the reciprocal impression

between enzyme and pH. In addition, squares of time, alcohol concentration, and pH were effective parameters for extraction efficiency.

The optimum condition suggested by the RSM analyzer was 35 ml/L enzyme concentration, 45 min processing time, 30% (v/v) alcohol in alcohol/buffer solution, and pH 7, leading to the absorbance of 11.28 (Table 5). Dye extraction was performed under optimum conditions, where the value of dye absorption (about 11.61) was closer to the predicted one by the model, as detailed in Table 5.

Table 5: Optimum condition for extraction process.

Response	Optimum extraction condition				Absorbance		Difference (CV)%
	Enzyme (ml/L)	Time (min)	Alcohol percentage(v/v)	pH	Experimental	Predicted	
Extracted dye	35	45	30	7	11.28	11.60	2.8

3.4. Colorimetric properties of dyed samples

The relationship between the K/S values of the dyed samples and the concentration of crude plus extracted madder roots is shown in Figure 4. It was found that K/S values increased with increasing the dye concentration from 10% to 60% (o.w.f) for wool dyed with crude madder and 1% to 30% (o.w.f) for samples dyed with UE-extracted madder dye. Higher dye adsorption was observed for the woolen yarn dyed with the extracted dye. In the dyeing, leaching, and diffusion of dye molecules take place simultaneously from crude materials into the dyeing solution. Thus, the dyeing process is longer due to the amount of dye available for adsorption depending on the leaching rate of dye from crude material. In dyeing with the extracts, all of the dye is available for adsorption from the beginning of dyeing, resulting in higher dye adsorption [24].

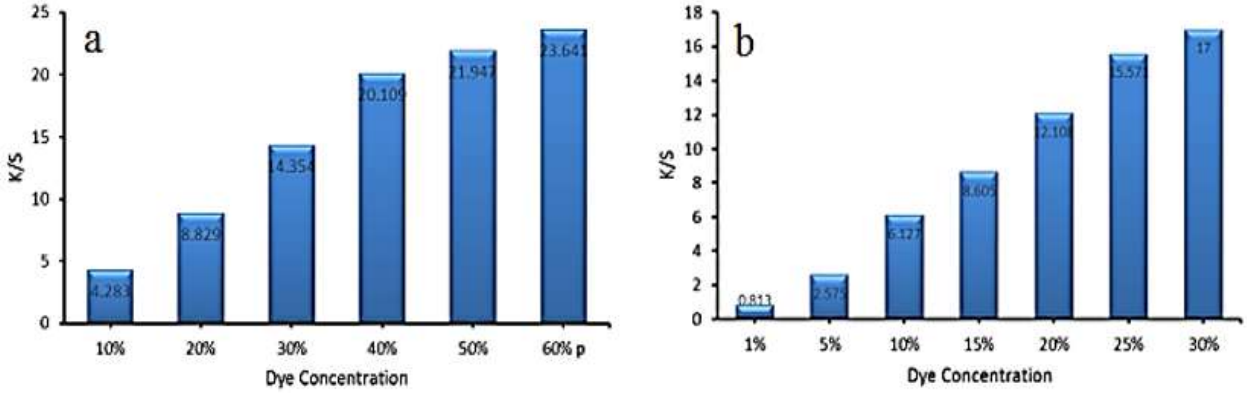

Figure 4: K/S values of dyed samples with aluminum sulfate (8%) a) crude madder, b) extracted dye by ultrasound/enzyme.

Both samples dyed with crude and extracted dye were in red-yellow hues (Figure 5), with the red hues being generally prevalent (a* > b*). For dyed yarns at 1/1 standard depth (40% crude madder and 25% UE-extracted method), it was observed that the lightness of dyed yarns was approximately the same. This shows better adsorption of dyes for the extracted dye. On the other hand, the hue angle of the wool samples dyed with crude madder was slightly smaller (30.33°) than that of the samples dyed with extracted madder (33.51°). A comparison of two samples indicated that by dyeing wool yarn with the extracted dye, the a* value was slightly affected, but the b* value shifted towards the blue region of the color space diagram. Differences in colorimetric data of the samples dyed with the extract and crude madder root can be related to the different energy gaps formed under dyeing conditions between the madder dye and metal ions, the nature of the metal ion in the chromophore, and the solution pH. Indeed, complexes with larger energy gaps lead to reddish hues, while complexes with shorter energy gaps result in bluish hues. Although the pH and mordant type were the same in this series, there was a slight difference between the extracted and raw materials that caused the color change. In addition, some colorless impurities in the raw material may have interacted with the

coloring materials, reducing the energy gap and creating a bathochromic effect as copigmentation [25].

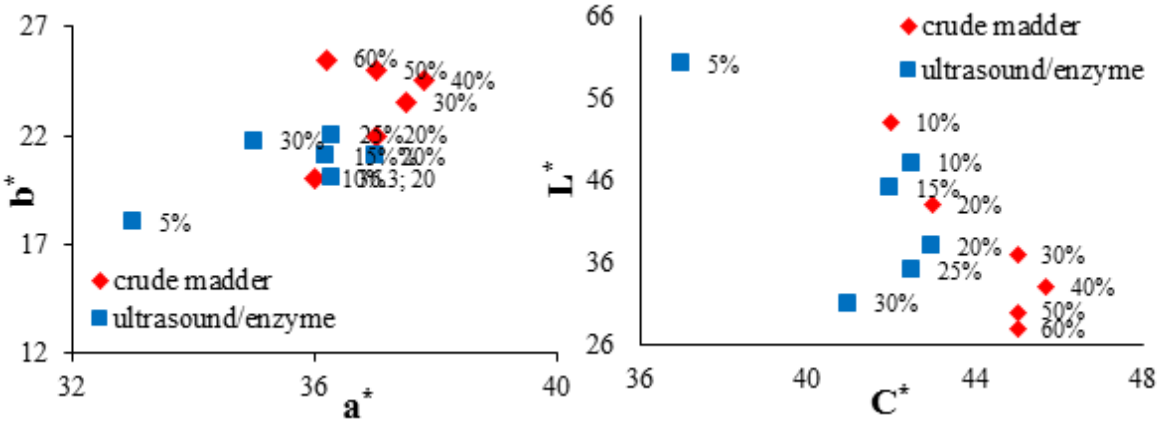

Figure 5: Color space diagram of dyed samples with crude madder and extracted dye by ultrasound/enzyme.

3.5. Effect of biomordants on colorimetric data of dyed wool yarns

A higher concentration of extracted dye would cause the color palette to shift towards the yellow-red quadrant (Table 6). The dyeing of wool yarns with Madder and extracted walnut has a low lightness value compared to other samples, with the hue of dyed samples moving to yellow. Myrtus communis mordant produced shades with high lightness where increasing the myrtus communis concentration led to color coordinates shift towards the green-yellow region. In the case of pomegranate mordanting, a* value diminished by increasing the concentration. Mordanting with amygdalus scoparia created diverse color gamut ranges with the lowest b* compared to other bio-mordants.

Table 6: The Effect of biomordants on color characteristics of yarns dyed with extracted madder.

Mordants	Concentration of mordants	UE assisted dye extract														
		10%					20%					40%				
		L*	a*	b*	C*	h°	L*	a*	b*	C*	h°	L*	a*	b*	C*	h°
Walnut hulls	5%	41.7	20.1	14.5	24.8	35.8	35.9	24.7	17.0	30.0	34.5	30.87	26.8	19.2	32.9	35.6
	10%	40.5	20.7	16.4	26.4	38.4	35.5	25.0	18.1	30.8	36.0	30.21	27.8	19.6	34.0	35.2
	20%	39.0	19.5	17.0	25.9	41.1	33.9	24.1	18.4	30.3	37.3	30.05	26.6	19.5	33.0	36.2
	40%	36.7	20.5	19.0	27.9	42.8	32.9	23.0	19.0	29.8	39.5	29.4	25.8	19.6	32.4	37.2
Pomegranate peel	5%	44.1	28.8	18.9	34.4	33.3	36.8	26.2	23.4	35.1	41.8	32.8	32.7	18.4	37.5	29.3
	10%	43.5	26.0	20.3	33.0	38.0	35.1	28.8	22.8	36.7	38.3	33.2	30.7	18.9	36.0	31.6
	20%	42.8	23.6	22.9	32.8	44.2	37.4	29.6	21.1	36.3	35.5	32.4	29.9	20.0	36.0	33.8
	40%	41.9	24.0	25.3	34.8	46.6	36.7	28.7	21.8	36.0	37.2	32.2	28.3	21.3	35.5	36.9
Myrtus communis	5%	52.0	22.2	17.1	28.1	37.6	52.0	22.2	17.1	28.1	37.6	41.4	28.0	17.0	32.7	31.4
	10%	50.8	21.4	19.6	29.0	42.5	45.6	25.7	18.7	31.8	36.1	40.1	27.6	18.6	33.3	34.1
	20%	49.9	20.0	21.7	29.5	47.3	45.8	24.3	20.3	31.7	39.9	40.3	25.4	19.4	31.9	37.3
	40%	47.1	19.6	23.1	30.3	49.6	44.1	22.0	21.6	30.8	44.4	39.4	25.3	20.6	32.7	39.2
Amygdalus scoparia	5%	47.3	24.9	13.7	28.5	28.9	44.8	26.7	14.1	30.2	27.9	41.5	29.5	14.3	32.8	25.9
	10%	45.9	23.9	15.3	28.4	32.6	43.6	25.3	14.9	29.4	30.5	37.3	27.3	15.3	31.3	29.3
	20%	44.2	24.5	16.9	29.8	34.7	40.2	26.3	16.5	31.1	32.2	36.4	26.6	16.3	31.2	31.5
	40%	41.9	25.4	18.6	31.5	36.3	39.5	26.7	18.2	32.3	34.3	36.5	27.3	17.4	32.4	32.5

Also, the dye adsorption of wool samples mordanted with pomegranate was higher than that of other mordanted samples. This can be related to the presence of ellagic acid as hydrolyzable tannin in extracted pomegranate, the coloring effect of pomegranate, and thus the synergistic effect with 40% extracted dye [26]. Meanwhile, Amygdalus scoparia

mordanting showed a very low color strength. The order of dye adsorption by wool samples was as follows: Amygdalus scoparia < Myrtus communis < gallnut < pomegranate, revealing the structural features of these colored biomordants. Figures 4 and 6 show that 10% bio-mordants such as walnut hulls and pomegranate with 20% extracted dye resulted in K/S of 13.5 and 10.3, respectively.

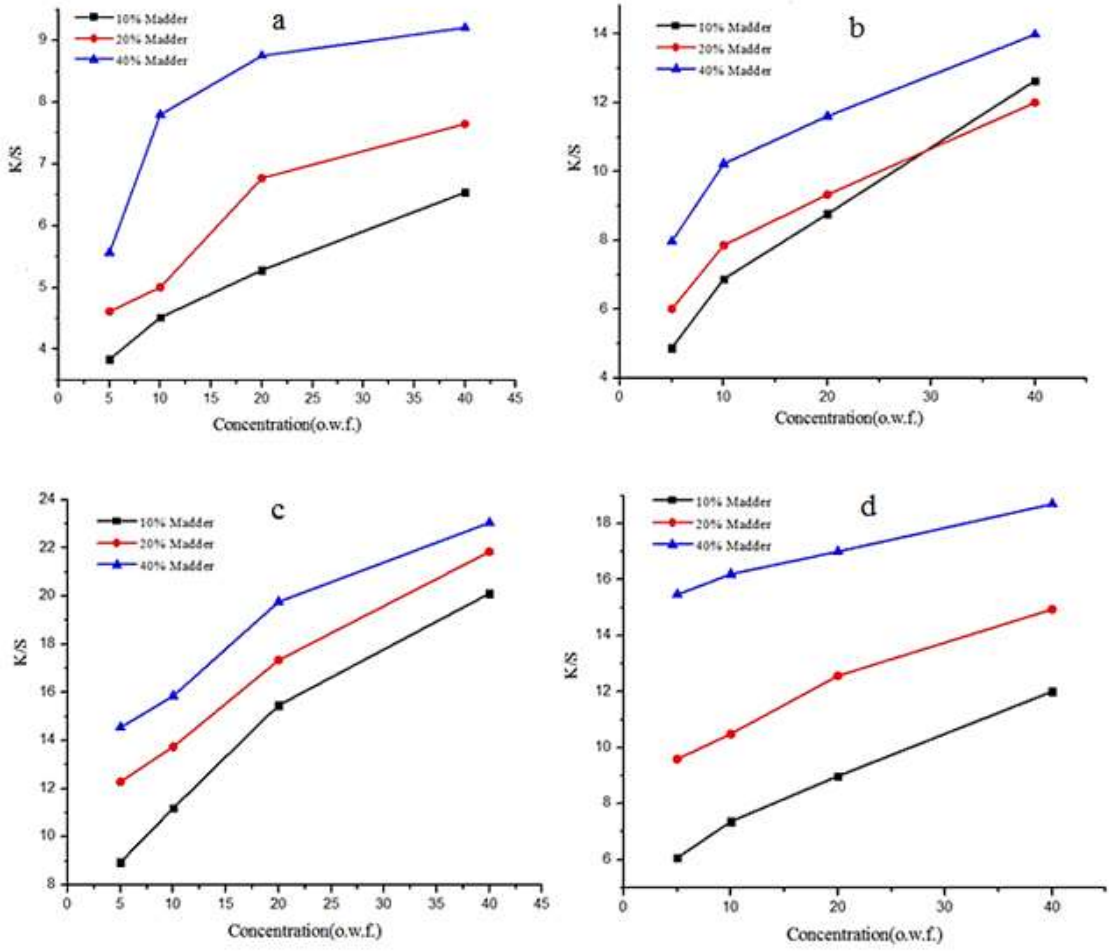

Figure 6: K/S values of yarns dyed with extracted dye, pre-mordanted with a) Amygdalus Scoparia,b) Myrtus Communis, c) pomegranate, and d) walnut.

3.6. Color fastness properties of dyed samples

The results of the washing fastness test involving staining on the adjacent multifiber and color changes of dyed samples are given in Table 7. Due to the high substantivity of madder dyes to such fibers, the degree of staining on the nylon and polyester as the adjacent components was greater than on other specimens, with low-level staining being expected for the adjacent cellulose [27]. Aluminum mordant forms an insoluble coordination complex with madder dyes during the dyeing process and leads to acceptable wash fastness (4.5) [28]. Walnut hulls, pomegranate peels, Myrtus communis leaves, and Amygdalus scoparia root are tannin-rich and full of phenolic hydroxyl groups with a high potential for binding to proteins [29]. Tannins in bio-mordants can form bonds such as hydrogen, ionic, and covalent bonds between the protein woolen fiber and madder dye [28, 29]. All dyed yarns have had good wash fastness properties.

Table 7: Fastness properties of dyed yarns with extracted dyes in 1/1 Standard Depth.

Mordant	Sample	Washing						Light	Rubbing	
		W	C	Ac	N	P	A		Wet	Dry
Walnut hulls		4-5	5	4-5	4	3-4	4	3	5	5
Pomegranate peel		5	5	5	4-5	3-4	4	3-4	5	5
Myrtus communis		4-5	5	4-5	4	4	4	4	4-5	5
Amygdalus scoparia		5	5	5	4-5	4	4	5	5	4
Aluminum sulfate		5	5	5	4-5	5	5	7	4-5	4-5

W: wool; C: cotton; Ac: acetate; N: nylon; P: polyester; A: acrylic

Table 7 indicates that pre-treatment of wool with aluminum sulfate and Amygdalus scoparia improved light fastness of madder-dyed yarns compared to other bio-mordants. Chromophores of madder can be protected against light damage in the presence of metal ions [30]. A coordination complex between aluminum sulfate, dye, and wool protects the chromophore by preventing photolytic degradation of the dye [31]. In the case of bio-mordants, auxochromic groups of the bio-mordant would lead to the dissipation of the energy of absorbed photons over the chromophore in the dye [32, 33]. Higher lightfastness was achieved for Amygdalus scoparia bio-mordanted wool samples. Table 7 reports excellent rubbing fastness (4-5) for wool dyed with the extracted dye in conjunction with metal mordant and bio-mordants.

4. Conclusion

An environmentally friendly and high-efficiency dye extraction from madder was performed using ultrasound irradiation and cellulase enzyme. The ultrasound-assisted extraction exhibited a high extraction yield (55%) at three steps after 8 min compared to classical extraction with water/alcohol solvent (48%) which is performed at five steps and needs an extraction time of 60 min for each step. This improvement originates from the development of acoustic cavitations through ultrasound irradiation, resulting in the enhanced mass transfer of dyes from the madder to the solvent. Furthermore, the enzyme-catalyzed hydrolysis of the cellulosic wall of the madder in the extraction process improved the dye extraction from the madder. RSM was successfully employed to optimize the operational variables, which were obtained as 35 ml/L enzyme concentration, 45 min, processing time, 30% (v/v) alcohol, and pH 7. The maximum

yield of 64% in one-step extraction can be obtained compared to the classical approach (48% yield in five extraction steps). The dyeing properties of wools with bio-mordants revealed that walnut and pomegranate can be used as renewable biomordants for wool dyeing with extracted madder dyes.

According to the findings, the extraction efficiency of the introduced method was higher than the conventional method. In addition, the extraction time, the number of extractions, and the extraction temperature were greatly reduced. Therefore, the new method may be cost-effective.

Based on the findings of this study, it can be concluded that research on natural dyes as a vast and biocompatible resource will continue soon. To improve extraction efficiency on an industrial scale, current techniques will be used in conjunction with enzymes as biological materials. Furthermore, novel natural dye sources for textile fibers will be shown.

5. References

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