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Optimization of ultrasound-assisted anthocyanin extraction from agricultural waste purple corn silk for multifunctional hemp finishes

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Abstract

The utilization of agricultural wastes for textile dyeing has gained popularity due to their safe and environmentally friendly properties, as well as the resource sustainability. Natural dye extraction is usually achieved through solvent extraction, which is time-consuming, requires a lot of solvent, and degrades desired compounds at high temperatures. Thus, this study investigated ultrasound-assisted anthocyanin extraction from agricultural waste purple corn silk as natural functional colorants for hemp finishes, given the numerous health benefits associated with anthocyanin. In order to optimize the extraction processes and evaluate the synergistic impact of these conditions, response surface methodology was implemented. The optimum conditions were 1:15 material-liquid ratio, 47% ethanol concentration, 60°C, and 20 min, yielding 240.25 mg·L^{-1} anthocyanin. The extracted anthocyanin was used for dyeing hemp fabrics and demonstrated satisfactory colorfastness, antibacterial action on both *S.aureus* and *E.coli*, with E.coli being more effective, and strong antioxidant (>80%). The dyed fabrics also exhibited their great UV shield (UPF value > 40+). Anthocyanin derived from purple corn silk could therefore be utilized as a natural functional color for medical and health products. Also, purple corn silk contains more anthocyanin than other natural sources, making it a promising natural anthocyanin resource in textile industry.

1. Introduction

The agriculture and food industries produce an enormous volume of organic waste as a result of the conversion of raw materials into finished goods [1]. According to the biorefinery concept, the utilization of agricultural wastes for natural textile dyeing has gained attention because of their safe and environmentally friendly properties, as well as the resource sustainability [2]. Mangosteen rind, corn cob, spent coffee, pomegranate rind, annatto seeds, and grape pomance are among the agricultural wastes that have been utilized to create natural colorants [3-8]. Purple corn (Zea mays L.), which was initially cultivated in Peru, has since become a globally cultivated crop owing to its high nutritional content and biological activities [9-11]. The abundance of anthocyanin and phenolic compounds in a purple corn contributes for its remarkable health. Purple corn possesses three main anthocyanins (Figure 1): Cyanidin-3-glucocide, Peonidin-3-glucocide, and Cyanidin-3-(6'-malonylglucocide), being accountable for the plant's coloring [10,12]. Many studies have shown that anthocyanins are strong antioxidants, have antibacterial properties, and protect against UV radiation [9,13]. Purple corn contains a high concentration of anthocyanin, particularly in the plant's husk, cob, and silk [9,11]. These three constituents of purple corn are regarded as byproducts and are discarded after being harvested and consumed. Nevertheless, corn cobs and husks have been utilized as a raw material for animal feed, charcoal, and compost. Similarly, purple corn silk can serve as an ingredient in the production of healthy products, including cosmetics, tea, and alternative medicines [14]. There have been a few studies using purple corncob to dyed natural fibers such as silk and cotton [4,15,16].

Conventional solvent extraction is the usual method used to extract natural anthocyanins. However, this conventional technique requires a significant amount of time and substantial solvent utilization (e.g., methanol, ethanol), and involves high temperatures, leading to the degradation of the desired compounds [17]. Since natural colorants are frequently deeply embedded in plant cell walls, the optimal extraction technique should be able to effectively promote the colorants' release and transportation into the solvent [18-20]. Emerging technologies like microwave and ultrasound extraction are being explored as possible substitutes for conventional techniques in the extraction of plant metabolites [21]. Ultrasound-assisted extraction (UAE) has received recognition for being an exceptionally efficient and environmentally sustainable extraction method [22]. The extraction process is significantly influenced by the simultaneous mechanical, cavitation, and thermal effects of ultrasound waves [17]. Ultrasound with a power range of 20 kHz to 100 kHz can be employed to intensify and enhance extraction processes [20]. As a result, the natural colorant textile industry can benefit economically and practically from the UAE, including reduced extraction times, lower solvent usage, increased extraction efficiency, improved quality of extracts, and enhanced extraction of heat-sensitive compounds with low yields. Backes *et al.* [23] conducted a comparative analysis of three extraction techniques (heat, ultrasound, and microwave)

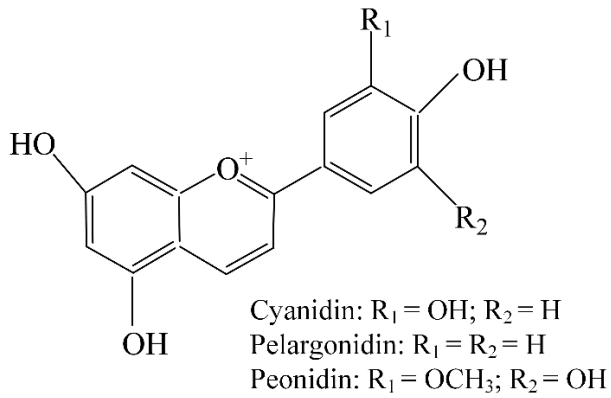

Figure 1. Basic chemical composition of anthocyanin

with the goal of recovering anthocyanin pigments and optimizing the extraction conditions from fig peel. The ultrasound extraction method proved to be the most efficient, resulting in the extraction of 19.4 mg of anthocyanin per one gram of residue weight. Microwave and heat extractions followed, in that order. Other studies have reported similar findings when conducting optimization studies with different natural plants [24,25]. Garcia-Castello *et al.* [18] compared the effectiveness of conventional solid-liquid extraction to UAE, and found that UAE produced 50% more total phenolic content and 66% more total antioxidant activity at lower temperatures and extraction times.

Despite cotton being the predominant cellulosic fabric, the climatic conditions in Southeast Asian countries and Thailand are unsuitable for commercial production of this material. Cannabis sativa, commonly known as hemp, is assigned to be an economic commodity in Thailand on account of its economic and environmental benefits. It is currently cultivated in the northern regions of the country. The composition of hemp fiber is as follows: 78% cellulose, 10% hemicellulose, 7% lignin, 3% pectin, 1% ester wax, and 2% water-soluble substance, [26]. The presence of cellulosic substances in hemp fiber contributes to its notable characteristics, including comfort, high moisture regain, and stability [27]. Enhancing the eco-friendly functional properties of hemp fabrics is essential to promoting their use in high-value products. Hence, the goal of this research is to examine the UAE of anthocyanin, a bioactive colorant derived from agricultural waste purple corn silk, to serve as a functionalizing agent and natural dye for hemp finishes. Response surface methodology (RSM) was employed to optimize the practical effects of UAE conditions, including ethanol content, ultrasound temperature, and ultrasound duration, in addition to their synergistic influence. The extracted anthocyanin was then applied to create multifunctional finishes on hemp fabrics. Validation of the optimal conditionals determined by RSM in practice was carried out through performance evaluations. These evaluations included tests for UV protection, antioxidant and antibacterial properties, as well as color fastness, in accordance with standard testing protocols. Zhou and Yang [8] investigated the extraction of anthocyanins from grape pomace, a byproduct of red wine, with ultrasound assistance. The optimum conditions were 1:15 material-liquid ratio, 47% ethanol concentration, 68°C, and 48 min, yielding 193.547 mg/100 g of anthocyanin extracted from grape pomace. Celli *et al.* [28] optimized the extraction of anthocyanins from haskap berries in the UAE process. The optimal conditions were as follows: a liquid-to-solid ratio of 25:1, 80% ethanol concentration, and an ultrasound bath temperature of 35°C for 20 min. These conditions resulted in a total anthocyanin content (TAC) of 22.73 mg·g^{-1} dry weight. As far as we know, no study has investigated the possibility of using anthocyanin derived from purple corn silk as a natural functional color for textiles.

2. Experimental

2.1 Materials

Purple corn silks obtained from the Royal project shop, Patumthani province. A plain weave hemp fabric with a density of 135 g·m^{-2} was purchased from Chaingmai province. In order to eliminate impurities from the fabric, a scouring process was done by using a nonionic detergent of 4 g·L^{-1} concentration at 50°C for 45 min. Subsequently, the fabric was washed and air dried. Aluminum potassium sulfate ($AlK(SO_4)_2$) and ethanol (CH_3CH_2OH) of AR grade were acquired from Sigma-Aldrich Co. Ltd.

2.2. Ultrasound-assisted anthocyanin extraction

Purple corn silks were cleaned thoroughly and thereafter dried at a temperature of 50°C for 2 h in an oven. They were then cut into multiple small pieces. Anthocyanins from purple corn silks were extracted using UAE in a 3L ultrasonic cleaning bath operating at 35 kHz (Sonorex DIGITEC DT 102 H-RC), as shown in Figure 2. The most effective solvent for extracting anthocyanin from purple corn silk was determined to be a combination of ethanol and water [8,13].

RSM was utilized to identify the most effective combination of UAE process factors for achieving the highest anthocyanin yield from purple corn silks. RSM is a highly efficient tool for optimizing operational variables to enhance bioactive compound extraction processes [5,7,8,20,29]. The process factors in the UAE, which were ethanol concentration, ultrasound temperature, ultrasound time, and, were determined using Box-Behnken design (BBD) coupled with RSM. BBD is a nearly rotatable second-order RSM class established from incomplete 3^3 factorial designs [30]. BBD uses fewer experiments to create higher- order response surfaces than a conventional factorial technique, making it more efficient than three- level complete factorial designs. Preliminary screening tests had been performed to identify the optimal range of UAE factors (Table 1). For each UAE experiment, 7 g of purple corn silk had been mixed into various ethanol solutions at a 1:15 material-liquid ratio. Afterward, the solutions that were extracted were filtered using Whatman No. 1. The pH differential method, as detailed in reference [29], was subsequently employed to determine the TAC of the extracts.

Figure 2. Ultrasound-assisted anthocyanin extraction.

Table 1. BBD experimental design levels

Symbols	Factors	Lower level	Upper level	Symbols
X	ultrasound temperature (°C)	55	65	X
Y	ultrasound time (min)	10	30	Y
Z	ethanol concentration (%)	40	60	Z

Table 2. Classification of UV protection effectiveness (AS/NZS 4399).

UV protection efficiency	Calculated UPF	UVR transmittance (%)
Excellent	40 to 50+	less than 2.5
Very Good	25 to 39	4.1 to 2.6
Good	15 to 24	6.7 to 4.2

2.3 Optimization of UAE process

To analyze the effect of UAE process factors on TAC, BBD was utilized, and the design consisted of 16 experiments shown in Table 3, with four replicates in the center point and two replicates in each experiment using Minitab software (version 20). In RSM, a response surface plot illustrates the mathematical correlation between experimental variables (X) and a response Y using a quadratic regression model, as represented by Equation (1).

$$Y = c_0 + \sum_{i=1}^{3} c_i X_i + \sum_{i=1}^{3} c_{ii} X_i^2 + \sum_{i=1}^{3} c_{ij} X_i X_j \quad (1)$$

Where Y is response function, X_i, X_j are coded variable values, and c_0, c_i, c_{ij}, and c_{ii} are intercept, linear, interaction, and quadratic coefficients, respectively.

An ANOVA was used to assess the statistical significance of each experimental variable in the predictive model. A *p*-value below the threshold of 0.05 was deemed to possess statistical significance, hence signifying statistical significance at a confidence level of 95%. The optimal settings for UAE process were then established by examining the response surface contour plots and solving the regression model equation.

2.4 Dyeing process for hemp fabric

Scoured hemp samples were treated with the optimal anthocyanin extract solution using infrared dyeing equipment for 60 min at 65°C through an exhaust method with a 1:30 material-to-liquor ratio. Both direct and simultaneous mordanting dyeing processes were carried out. In simultaneous mordanting dyeing process, the dyebath was supplemented with potassium alum ($AlK(SO_4)_2$) as a mordant, with a concentration of 5% owf. The dyed fabrics were then rinsed with distilled water and air dried.

2.5 Color evaluation

The spectrophotometer (GretagMacbeth LLC, USA) was employed to quantify colorimetric properties of the fabric samples. The spectrophotometer was equipped with an illuminant D65, specular and UV included, and a 10° standard observer. Each test was performed three times, with the mean value recorded. The Kubelka-Munk Equation (Equation (2)) had been utilized for calculating the color strength (K/S).

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

Where K, R, and S denote absorption coefficient, reflectance at the highest absorption wavelength, and scattering coefficient, respectively.

The colorimetric values are represented by CIELAB coordinates (*L*, a*, b*, h°, and C**). In this system, *L** denotes brightness, with 100 indicating white and 0 indicating black. The green-red axis is denoted by the *a** coordinate, where negative values signify green and positive values signify red. The *b** coordinate denotes the blue-yellow axis, where negative values indicate blue and positive values indicate yellow. Additionally, *C** and *h°* represent chroma and hue angle, respectively.

The evaluation of color fastness against crocking, light, and washing was performed using the AATCC 8-2007, ISO 105-B02:2013, and ISO 105-C01:2013, respectively.

2.6 Measurement of UV protection efficiency

Ultraviolet radiation (UVR) transmittance and ultraviolet protection factor (UPF) of fabric samples were assessed using the CamSpec M550 SPF Spectrophotometer (Spectronic CamSpec Ltd., UK) following AS/NZS 4399 test method (Table 2). Higher UPF value indicates that textiles have a greater ability to protect against UV rays. The UPF value was computed using Equation (3):

$$UPF = \frac{\sum_{290\ nm}^{400\ nm} R_\lambda S_\lambda \Delta\lambda}{\sum_{290\ nm}^{400\ nm} R_\lambda S_\lambda T_\lambda \Delta\lambda} \quad (3)$$

Where T_λ, R_λ, S_λ, and $\Delta\lambda$ are specimen's average spectral transmittance, relative erythemal spectral effectiveness, solar spectral irradiance, and wavelength interval (nm), respectively.

Equation (4) and Equation (5) denote the mean transmittance values in the UVA range of 315 nm to 400 and UVB range of 290 nm to 315 nm, respectively.

$$T(UVA) = \frac{\sum_{315\ nm}^{400\ nm} T_\lambda \Delta\lambda}{\sum_{315\ nm}^{400\ nm} \Delta\lambda} \quad (4)$$

$$T(UVB) = \frac{\sum_{290\ nm}^{315\ nm} T_\lambda \Delta\lambda}{\sum_{290\ nm}^{315\ nm} \Delta\lambda} \quad (5)$$

2.7 Measurement of TAC

The quantification of TAC was performed by applying the pH differential method, as stated in [29]. This method involves measuring absorbance at pH 1.0 and pH 4.5 to identify any structural alterations in the chemical forms of anthocyanin. Two distinct standard buffer solutions were created. The solution (pH = 1) was made by mixing 25 mL of a potassium chloride solution with a concentration of 0.2 mol·L^{-1} and 67 mL of a hydrochloric acid solution with a concentration of 0.2 mol·L^{-1}. Another solution (pH = 4.5) was prepared by combining 6 mL of hydrochloric acid with a concentration of 1 mol·L^{-1}, 10 mL of sodium acetate with a concentration of 1 mol·L^{-1}, and 9 mL of deionized water. The TAC had been determined and expressed in relation to the cyanidin 3-glucoside equivalent, as shown in Equation (6).

$$\text{TAC (mol·L}^{-1}) = \frac{A \text{ x } Mw \text{ x } DF \text{ x } 1000}{\mathscr{E} \text{ x } L} \quad (6)$$

Where A = absorbance value [(Aλmax – A700)pH1.0 - (Aλmax – A700)pH4.5], Mw of cyanidin 3-glucoside = 449.2 g/mol, $\mathscr{E}$ = 26,900 (molar extinction coefficient of cyanidin 3-glucoside , DF = dilution factor, and L = cuvette's path length.

2.8 Evaluations of antibacterial and antioxidant properties

The antibacterial effectiveness of the treated hemp fabrics regarding *E. coli* (ATCC 25922, gram-negative) and *S. aureus* (ATCC 6538, gram-positive) was evaluated using a qualitative test procedure in accordance with AATCC 147-1998 [3].

The antioxidant activity of treated hemp fabrics is analyzed utilizing the DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging method, as reported in [20]. Briefly, a fabric sample weighing 500 mg was immersed in a 30 mL solution of 0.10 mM DPPH methanol and incubated for 30 min in the dark. The solution's color shift from dark violet to pale yellow was noticed, and its absorbance at 517 nm was detected using a UV spectrometer. This study used ascorbic acid as a positive control. The percent antioxidant activity had been computed via Equation (7).

$$\text{Antioxidant activity (\%)} = \frac{U-T}{U} \text{ x } 100 \quad (7)$$

Where U and T denote absorbances of dark-incubated solutions for the untreated and treated specimens, respectively, at a wavelength of 517 nm.

3. Results and discussion

3.1 Extraction of TAC

To optimize the process conditions for extracting the natural colorant anthocyanin with ultrasound assistance, three-level and three-factor BBD was designed as the foundation for conducting single-factor extraction tests (Table 3). The BBD experimental results were examined using the software Minitab and fitted to a quadratic regression model denoted by the following equation:

$$\text{TAC (mg·L}^{-1}) = 148.41 + 3.083*X + 0.255*Y + 0.075*Z - 0.001*X*Y + 0.006*X*Z - 0.0005*Y*Z - 0.0284*X^2 - 0.0052*Y^2 - 0.0005*Z^2$$

Where, X is ultrasound temperature, Y is ultrasound time, and Z is ethanol concentration.

Table 3. Corresponding response of BBD experiments.

No.	Experimental variables			TAC
	Temperature (X, °C)	Time (Y, min)	Ethanol Conc. (Z, %)	(mg·L^{-1})
1	55	20	40	212.96
2	60	20	50	237.51
3	55	20	60	211.91
4	65	10	50	199.48
5	55	10	50	197.46
6	55	30	50	223.29
7	65	20	40	201.25
8	65	30	50	213.22
9	60	30	60	208.32
10	60	20	50	237.60
11	60	10	60	213.41
12	60	30	40	201.88
13	65	20	60	199.51
14	60	20	50	238.26
15	60	10	40	213.79
16	60	20	50	238.97

Table 4. ANOVA for fitted regression model.

Effect	Sum of square	Degree of freedom	Mean square	F-value	p-value
Linear	1.482	3	0.494	87.23	0.000
Square	3.231	3	1.077	190.18	0.000
Interaction	0.434	3	0.145	25.53	0.002
Model	4.074	9	0.453	79.94	0.000
X-Temp	0.086	1	0.073	15.16	0.011
Y-Time	0.993	1	0.993	175.47	0.000
Z-Ethanol	0.719	1	0.719	126.98	0.000
XY	0.002	1	0.002	0.38	0.564
XZ	0.422	1	0.422	74.55	0.000
YZ	0.009	1	0.009	1.66	0.254
X2	1.855	1	1.855	327.58	0.000
Y2	1.004	1	1.00393	177.28	0.000
Z2	0.841	1	0.84121	148.55	0.000
Lack-of-Fit	0.028	3	0.009		
Total	**4.103**	**15**			

This study utilized ANOVA to assess the fit of the quadratic regression model (Table 4). The regression model is fitted when the *p*-value is < 0.05, which indicates statistical significance with a 95% confidence level. The statistical analysis (Table 4) revealed that the variables fit the regression equation well, as illustrated by a p-value below 0.05. Additionally, the correlation coefficient (R^2) showed a satisfactory value of 0.9351, meaning that 7% of the variance could not be accounted for by the present model. A regression model with R^2 closer to 1 suggests a greater level of quality. The statistical analysis of Table 4 also reveals that the linear coefficients (X, Y, and Z), interaction coefficient (XZ), and quadratic coefficients (X^2, Y^2, and Z^2) all exhibit statistical significance with *p*-value < 0.05. The F value indicates that the independent variables for the UAE of TAC in the prediction model were significant in the following order: ultrasound time > ethanol concentration > ultrasound temperature. The regression model analysis reveals that the independent variables' main, interaction, and square effects are statistically significant

3.2 Extraction efficiency surface plots

The optimum conditions of the UAE process were examined in order to reach the highest content of anthocyanin. Three-dimensional diagrams and contour plots were created using the complete equation model to examine the correlation between UAE conditions and total anthocyanin yield. Figure 3 demonstrates that TAC increased as ultrasound temperature increased while keeping ultrasound time constant. Additionally, the anthocyanin content increased with increasing ultrasound times. By elevating the temperature, the purple corn silk exhibits enhanced absorption, allowing the solvent to enter and disperse within the plant's microstructure. The efficiency of plant extraction is enhanced by two factors: increased cell wall vulnerability and reduced solvent viscosity at high temperatures [20]. However, the extracted anthocyanin is susceptible to chemical degradation, resulting in a color change at higher temperatures (above 65°C). This is attributable to the glycoside bond within its chemical structure. First-order kinetics is the main mechanism observed in a thermo-degradation of anthocyanin [31]. TAC extraction was found to be influenced by both the ultrasound temperature and time. The anthocyanin extract increased due to a higher concentration of anthocyanin being dissolved in the solvent as the ultrasound time increased. At the moderate level of temperature and time in the UAE conditions, purple corn silk exhibited a higher yield of anthocyanin extraction. The contour plot shows that the optimal ultrasound temperature is between 58.5°C and 61°C, while the optimal ultrasound time is between 16 and 22.5. Figure 4 illustrates the impact of ethanol concentration and ultrasound temperature on TAC yield. The TAC exhibited a substantial increase with higher ethanol concentrations under a specific ultrasound temperature. Furthermore, TAC increased in correlation with the ultrasound temperature. The contour plot shows that the optimum ultrasound temperature is between 58.5°C and 61°C, while the optimum ethanol concentration is between 44% and 51%. Figure 5 presents the impact of ethanol concentration and ultrasound time on TAC yield. The TAC exhibited a substantial increase as the ethanol concentration increased under a specific ultrasound time, whereas the plot remained comparatively stable as the ultrasound time increased for a given ethanol concentration. It indicates that the ethanol concentration significantly impacted the TAC extraction. The contour plot shows that the optimum ultrasound time falls within the range of 16.5 to 23, while the optimum ethanol concentration falls within the range of 44 to 51. In summary, the response surface plots illustrated the outcomes that corresponded with ANOVA of the quadratic fitting equation.

The UAE of anthocyanin derived from purple corn silk was optimized using Minitab software. The optimum outcome was achieved with a material-liquid ratio of 1:15, a 47% ethanol concentration, a 60°C ultrasound temperature, and a 20 min ultrasound time. These conditions provided a predicted TAC of 244.62 $mg \cdot L^{-1}$. We then conducted the extraction experiment under optimum conditions, yielding an actual TAC of 240.25 $mg \cdot L^{-1}$. This yield closely approximated the predicted value of 244.62 $mg \cdot L^{-1}$, with a margin of error of 1.82%. Thus, the study's proposed model has been confirmed and validated. Consequentially, the obtained anthocyanin colorants would be applied to finished hemp fabrics in order to assess their colorfastness and multifunctional properties.

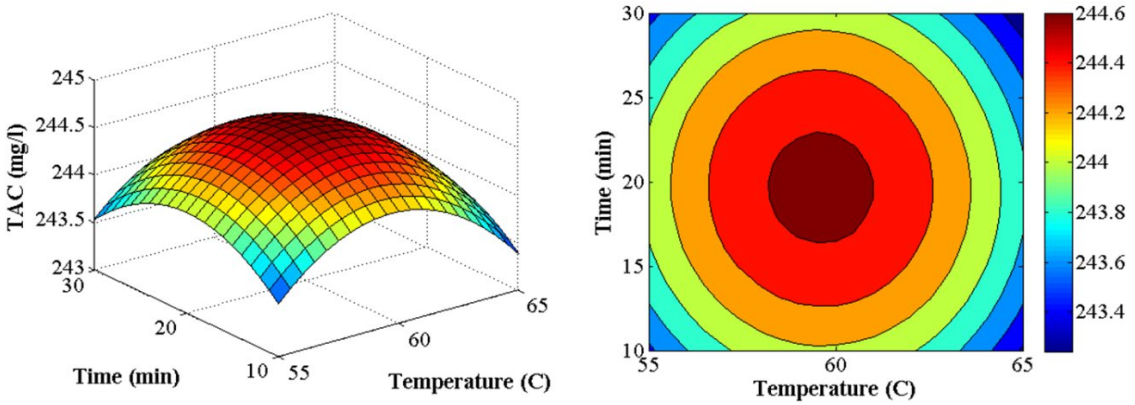

Figure 3. Impact of ultrasound temperature and time on TAC extraction.

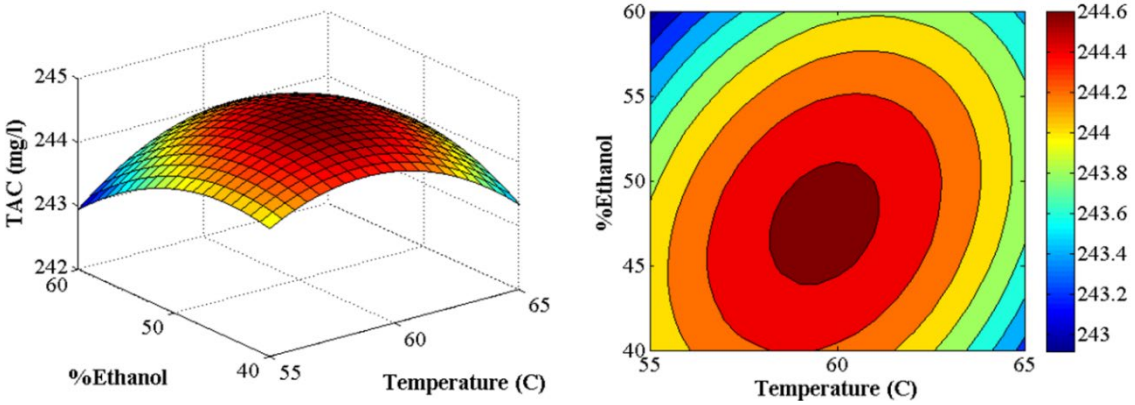

Figure 4. Impact of ethanol concentration and ultrasound temperature on TAC extraction.

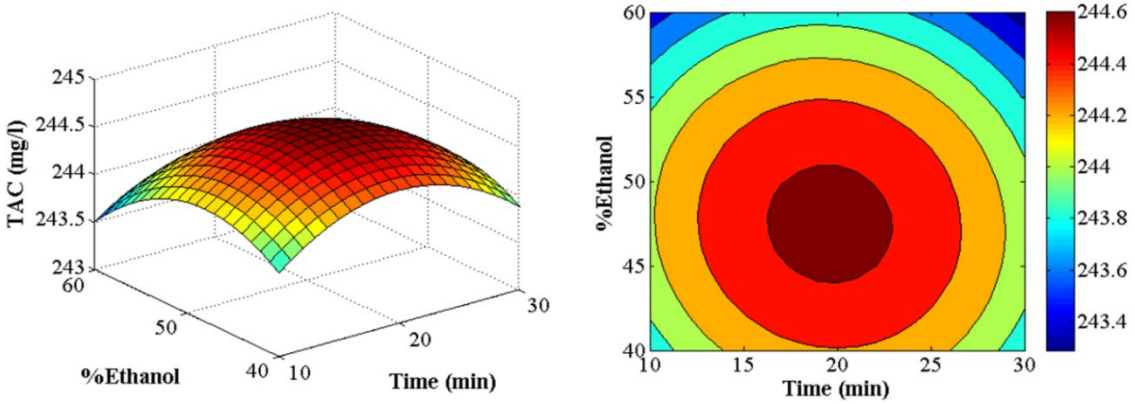

Figure 5. Impact of ethanol concentration and ultrasound time on TAC extraction.

3.3 Dyeing and colorfastness properties

Anthocyanin, a natural colorant extracted from purple corn silks with ultrasound assistance under optimal conditions, was used to dye hemp fabfics. Our study examined CIELAB colorimetric values, color strength, and colorfasgness of the treated hemps, as shown in Table 5, L^* indicates lightness, with lower values indicating darker shades. The chromaticity coordinates a^* and b^* represent color attributes: $-a^*$ for greenness, $+a^*$ for redness, $-b^*$ for blueness, and $+b^*$ for yellowness. No substantial difference was observed between L^* of treated hemp fabric using mordant dyeing method and that using direct dyeing method. However, a decrease in lightness noticed in the mordant dyeing method can be attributed to a greater absorption of dye onto the fabric, leading to a deeper shade compared to the direct dyeing method. The a^* and b^* values shifted the perception of redness and blueness from direct dyeing method to mordant dyeing method. An additional benefit of employing alum as a mordant in natural anthocyanin dyeing is that it enhances color strength of the treated fabric without altering the dye's original hue. As a result, our mordant-dyed hemp fabric retain its natural tone appearance. The color strength values of treated hemp were 3.07 for mordant dyeing and 2.13 for direct dyeing. Applying mordant dyeing resulted in a 44% increase over the direct dyeing method.

Table 5. Colorimetric values and Color strength.

Dyeing method	Colorimetric value					K/S	Image
	*L**	*a**	*b**	*h°*	*C**		
Untreated	88.08	0.60	7.41	85.38	7.44	0.16	
Direct dyeing	51.47	10.85	-0.55	357.10	10.87	2.13	
Mordant dyeing	48.31	20.41	-1.44	355.96	20.46	3.07	

Table 6. Washing fastness test.

Dyeing method	Color change	Color staining					
		Cotton	Acetate	Wool	Polyester	Acrylic	Nylon
Direct dyeing	2-3	4-5	4-5	4-5	4-5	4-5	4-5
Mordant dyeing	3-4	4-5	4-5	3-4	4-5	4-5	4-5

Table 7. Color fastness to light and crocking.

Dyeing method	Light	Crocking	
		Dry	Wet
Direct dyeing	3	3	2-3
Mordant dyeing	3-4	4	3-4

Table 8. UV protection and antioxidant activity.

Sample	UPF	UPF protection class	Transmittance (%)		Antioxidant activity (%)
			UV-A	UV-B	
Untreated	13.9	No class	15.05	9.26	-
Direct dyeing	43.6	Excellent	2.8	2.4	84.6
Mordanting dyeing	47.5	Excellent	2.0	1.8	80.2

Table 6 displays the outcomes of the washing fastness test. The evaluation of washing fastness was performed by examining color staining on multifiber fabrics and color change. The color change and color straining washing fastness achieved using the mordant dyeing method were rated as 3 to 4 (fair to good) and 4 to 5 (good to excellent), respectively. The color change improved by 1.5 grade compared to dyed fabric using direct method. The crocking fastness of dyed fabric using mordant method was assessed as 2 to 3 (poor to fair) when wet but 4 (good) when dry, representing a one-grade improvement over the direct method (Table 7). This is because polar solvents increased the probabilities of color loss in fabrics when rubbed, particularly when they were wet. The light fastness of fabrics treated using mordant and direct dyeing methods was rated as 3 to 4 (poor to fair), as shown in Table 7. Treated hemp fabrics dyed with mordant showed no significant difference in colorfastness to light compared to those dyed directly. Natural dyes are characterized by their poor light color fastness, rarely attaining a rating of 5 (good), with the exception of alizarin and lac, which contain an anthraquinone chromophoric structure [4,6].

Color stability of the treated fabrics is determined by colorfastness assessment. Our findings demonstrated that using alum as a non-toxic metal mordant enhanced the washing and rubbing colorfastness of hemp treated with anthocyanin extract. This is because alum mordant contains aluminum ions, which can interact with -OH groups in anthocyanin and -C=O and -OH groups in hemp fiber (Figure 6), allowing the natural anthocyanin to adhere to the hemp fabric and enhance colorfastness.The low light fastness, however, would restrict the outdoor application of anthocyanin-dyed cellulose fabric. The UV protection, antioxidant, and antibacterial properties of the hemp treated with anthocyanin extract would be further evaluated for their practical use.

3.4 UV protection and antioxidant properties

Nowadays, the ozone layer depletion is accelerating, causing an increase in the strength of sunlight radiation, which poses a serious threat to humans. Textiles act as a crucial barrier between the human body and the surrounding atmosphere, providing ultraviolet (UV) protection through several factors such as finishing chemicals, fiber composition, and fabric structure [32]. Some specific natural dyes possess the capacity of absorbing UV radiation, enabling them to obstruct UV light while also providing color. The efficacy of UV

protection provided by dyed hemp fabrics with extracted anthocyanin is assessed using the percentage transmittance of UV and the UPF. Percentage transmittance quantifies the proportion of UVR reaching the body, while UPF is a rating system that assesses the efficacy of the fabric shield against UV rays. A higher UPF value indicates the fabric offers better shielding against UV rays. Level of UV protection is affected by a variety of factors, including the fiber used. Natural fibers typically provide little protection. The construction of the fabric also plays a role, as thicker and denser fabrics provide more UV protection. Sized and unbleached fabrics provide more UV protection than desized and bleached fabrics. Moreover, the dyeing process has an impact on UV protection, as darker colors enhance it [33]. Table 8 shows the UPF value as well as percent transmittance of UVA and UVB for dyed hemp and untreated fabrics. The findings indicate a substantial difference between dyed and untreated hemp textiles. The untreated hemp had a UPF value of 13.9. Hence, in the absence of specific treatment, pristine hemp cannot offer sufficient protection against UV rays. This is because hemp's fiber structure lacks carbon-carbon double bonds, or UV-absorbing molecules [27]. Hemp fabrics treated with the colorant anthocyanin contain functional groups (flavonoid and polyphenol) that absorb UV radiation, providing better UV protection than untreated fabrics [31]. This is supported by the lower proportion of UV transmission and the higher UPF values. The dyed hemp also exhibited the greatest UPF (40+) rating, indicating superior UV protection. Furthermore, dyeing with a metal mordant (Al mordant) improves color depth by enhancing dye-fiber affinity, leading to a higher UPF value.

The presence of antioxidants in fabrics that directly touch the human body has generated interest in developing clothing that promotes health. Research studies have demonstrated the efficacy of anthocyanin in radical scavenging, suggesting its potential as a natural antioxidant to boost the antioxidant activity of textiles [34]. Therefore, our study was to assess the antioxidant capacity of dyed hemp with anthocyanin extract using the DPPH test. After being treated with anthocyanin extracted from purple corn silk, the hemp fabric demonstrated 84.6% antioxidant activity. This could be explained by the anthocyanin extract's ability to scavenge radicals, as it contains active hydrogen groups in polyphenolic compounds [7,22,31]. Dyed hemp treated with mordant had lower antioxidant properties than dyed hemp without mordant, possibly due to a decrease in free hydroxyl groups caused by the interaction of hemp, metal ions, and dye molecules.

3.5 Antibacterial activity test

An agar-well diffusion method was employed to assess the antibacterial activity, which was then quantified as zone of inhibition according to AATCC Test Method 147. The antibacterial effectiveness of tetracycline antibiotics, hemp treated with anthocyanin extract, and unadulterated hemp on *S.aureus* and *E.coli* is depicted in Figure 7. While the tetracycline antibiotics exhibited a more pronounced impact, the dyed hemp demonstrated superior efficacy in impeding bacterial proliferation when compared to the untreated hemp. The direct dyed hemp exhibited clear inhibition zones, with diameters of 1.2 mm for *E. coli* and 0.8 mm for *S.aureus*. Similarly, the mordant dyed hemp displayed clear inhibition zones measuring 0.9 mm against *S.aureus*

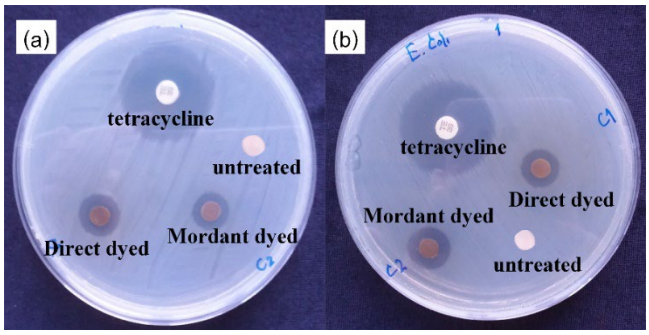

Figure 7. Antibacterial activity regarding: (a) *S.aureus* and (b) *E.coli.*

and 1.2 mm against *E. coli.* The treated hemps exhibited potent antibacterial action on both *S.aureus* and *E.coli*, with the latter having a greater effect. This was explained by the presence of large amount of phenolic compounds in anthocyanin extracted from purple corn silk. Previous research has demonstrated that natural polyphenolic extracts and polyphenols contain strong antimicrobial activities against pathogens [35]. The findings of our study indicate that the antibacterial efficacy of hemp treated with anthocyanin extract derived from purple corn silk was unaffected by alum mordanting. The findings of our examination align with the prior research conducted by [36-38].

4. Conclusions

The UAE is an environmentally friendly and highly efficient technique for anthocyanin extraction from purple corn silk. RSM optimized UAE conditions to achieve the maximum TAC, resulting in a TAC of 240.25 mg·L^{-1} at 60°C ultrasound temperature, 20 min ultrasound time, 47% ethanol concentration, and 30 Hz ultrasonic power. Purple corn silk has a high anthocyanin content when compared to other natural anthocyanin sources, making it a promising new natural anthocyanin resource in the textile industry. The colorant anthocyanin extract was then utilized to dye the hemp fabrics, and the mordant dyeing produced a 44% greater K/S value than the direct dyeing. The dyed fabrics demonstrated satisfactory color fastness, good antioxidant and antibacterial activities, and strong UV protection, which were attributed to the chemical structure of natural anthocyanin. Given its appealing properties and economic value, hemp treated with the natural anthocyanin extracted from agricultural waste purple corn silk can be utilized to create medical, healthy, and hygiene products. In conclusion, the utilization of UAE presents a highly promising concept as an alternative approach for extracting natural anthocyanin. This method has the potential to decrease dependence on solvents as well as reduce time and energy consumption. Hemp, when treated with the natural anthocyanin derived from agricultural waste purple corn silk, can be used to produce medical and healthy products due to its attractive characteristics and economic worth.

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