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Original paper

Optimization of conventional and ultrasound-assisted extraction of Paeonia officinalis anthocyanins, as natural alternative for a green technology of cotton dyeing

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Abstract Plant pigments gained popularity over synthetic dyes because of safety issues regarding the impact on health and environment. The present paper deals with optimization of anthocyanins extraction from *Paeonia officinalis* L. flowers and their application in cotton eco-dyeing processes. The results on extraction and mathematical models indicate improved extraction by ultrasonication at high solvent/solid ratio (50/1) compared to that by maceration. Among the investigated extraction solvents, the most efficient was 70% ethanol. Cotton dyeing was performed *via* conventional procedure and ultrasonication, in the presence and absence of classic mordant (copper sulphate) and biomordants (tannic and citric acids). A successful dyeing was obtained according to ATR-FTIR analysis of mordanted dyed samples. The colour properties of dyed cellulosic substrates as determined by CIELAB system showed higher redness values and large positive differences in chroma in samples dyed with peony extract by exhaustion in presence of high concentration of citric acid.

Keywords Peony, anthocyanins, ultrasounds, dyeing, cotton, mordant.

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Introduction

Natural dyes have been gaining popularity compared to synthetic dyes in textile industry, based on some evidence for potential toxic effects of latter ones with negative impact on health and environment (POKHARIA, SINGH, 2015).

Natural dyes extracted predominantly from plant sources may constitute a good alternative for cellulosic and protein substrates as they are biodegradable and safe, providing additional properties, such as antimicrobial. Numerous studies have been reported on using biopigments extracted from skin, bark, leaves, flowers and fruits of several plants (red onion, Eucalyptus, Mahonia, henna, green tea, Hibiscus, marigold, blackcurrant, etc.) (VANKAR, SHUKLA, 2019; COMAN, OANCEA, VRÎNCEANU, STOIA, 2014; COMAN, OANCEA, VRÎNCEANU, 2016).

The main drawback of dyeing with biopigments is the poor fastness of dyed textile products, but attempts have been done to overcome it by using mordants (HWANG, KIM, LEE, KIM, 1998) and more recently by using beta-cyclodextrin polymers to adsorb the dyes (CRINI, 2003). However, metallic salts commonly used as mordants showed negative environmental effects in their turn, so that research has been achieved for developing eco-friendly mordants such as oil products, tannins, tannic acid, tartaric acid and plant extracts (IŞMAL, YILDIRIM, 2019). Dyeing technologies with natural extracts are based either on conventional methods or new alternative technologies (ultrasonic, plasma, microwave, enzymatic, nanotechnology) (AHMED, EL-SHISHTAWY, 2010). The ultrasonication procedure provides an enhanced dye penetration, being preferred because of lower temperatures and time required as well as reduced dye quantity and electrolyte (KASIRI, SAFAPOUR, 2014).

Appropriate extraction of natural dyes in order to achieve high amounts of compounds from selected plant materials, and proper dyeing technologies are important for developing innovative value-added materials.

Among natural dyes, anthocyanins have been intensively studied based on their colouring properties and health benefits (WROLSTAD, 2006). Several conventional and non-conventional extraction technologies of anthocyanins have been previously described, efficiency being confirmed for ultrasound-assisted extraction (UAE) (HANDA, KHANUJA, LONGO, RAKESH, 2008). Optimal process parameters, such as solvent type and concentration, solvent-to-solid ratio, temperature and time must be determined for each experiment in order to get high extraction yield of bioactive compounds or to achieve the lowest energy consumption. Usually, addition of small amounts of acids to the extraction solvent improves the extractability of anthocyanins by stabilization of the coloured flavylium cation (GIUSTI, WROLSTAD, 2001).

This study was designed to describe the optimal extraction conditions for high recovery of anthocyanins from peony (*Paeonia officinalis* L.) flowers under conventional and UAE techniques, and to further apply the crude extract in a cotton eco-dyeing process. Cotton samples were dyed with the natural extract *via* conventional procedure and ultrasonication, in the presence and absence of classic mordant (copper sulphate) and biomordants (tannic and citric acids). The colour properties were investigated by determination of CIELAB values. ATR-FTIR analysis was also performed.

Material and Methods

Plant material and chemical reagents

Petals of common cultivated red peony (*Paeonia officinalis* L.) were collected at full bloom stage from Sibiu area, Romania. Samples were air-dried at 25ºC. Moisture content was determined using the moisture analyzer (MAC 210/NP Radwag, Poland).

Chemical reagents of analytical grade without further purification were used.

Extraction procedures and assay of total anthocyanins

Peony samples were grounded using the knife mill (Grindomix GM 200, Retsch, Germany). Extraction was done by a two-method approach. Three extraction solvents were tested: distilled water, ethanol and acidified ethanol solution.

Conventional extraction

Anthocyanins were macerated in 70% (V/V) ethanol solution and distilled water, respectively, at 4ºC, for 2 hours, at three different solvent/solid ratio (48/1, 24/1 and 12/1) and three different temperatures (30ºC, 40ºC and 50ºC).

Ultrasound-assisted extraction (UAE)

The experimental design for the applied UAE process using two solvents (ethanol and acidified ethanol) is shown in Table 1.

Table 1. Experimental data with different combinations of solvent/ solid ratio, extraction time and ultrasonic amplitude.

Experiment run	Solvent/ solid ratio	Ultrasonic extraction time (min)	Ultrasonic amplitude (%)
E1	50/1	10	50
E2	50/1	10	50
E3	50/1	20	70
E4	50/1	20	70
E5	50/1	30	50
E6	50/1	30	70
E7	40/1	10	50
E8	40/1	10	50
E9	40/1	20	70
E10	40/1	20	70
E11	40/1	30	50
E12	40/1	30	70

Anthocyanins were ultrasonically extracted in 70% (V/V) ethanol solution and acidified ethanol with 1% citric acid using an ultrasonic device (Sonifier SLPe-150, Branson,

USA) of 150 W power and 40 kHz frequency, equipped with a transducer. High solvent/solid ratios were used (50/1 and 40/1) as determined from optimal conditions under maceration procedure. The solvent-sample mixtures were irradiated in pulsed mode for three predetermined extraction times (10, 20 and 30 minutes) and two ultrasonic amplitudes (50 and 70%). Control samples prepared by maceration at 4ºC, for 24 hours, at 50/1 and 40/1 solvent/solid ratios were additionally investigated for the investigated extraction solvents (ethanol and acidified ethanol).

After extraction, samples were centrifuged at 8000 rpm at 4ºC for 10 min using the refrigerated centrifuge (Universal 320, Hettich, Germany).

The content of total anthocyanins in crude extracts was determined spectrophotometrically by the pH differential method (GIUSTI, WROLSTAD, 2001). Values were expressed as mg cyanidin-3-O-glucoside 100 g^{-1} DM.

Mordanting and dyeing procedures of cotton with peony extract

Samples of 100% cotton fabrics with specific mass of 180 g m^{-2} were used for dyeing with the peony extract which proved higher recovery of anthocyanins through the previously described extractive experiments.

Experimental data on dyeing of cotton samples with *Paeonia officinalis* L. extract are given in Table 2.

Two dyeing procedures have been applied: exhaustion and ultrasonication. Dyeing was performed with assistance of mordants in concentration of 3 and 5%, by immersion of cotton fabric samples in the dye bath. Copper sulphate (CS) was used as classic mordant, while citric acid (CA) and tannic acid (TA) as biomordants, at a fixed liquor ratio of 1:50. Dyeing by exhaustion was done at 80ºC for 15 minutes, while dyeing by ultrasonication was performed at r.t. for 15 minutes using the Elmasonic E Ultrasonic bench top bath.

Table 2. Experimental data on cellulosic fibers supports dyed with *Paeonia officinalis* L. anthocyanins extract

Sample	Description of cellulosic fibers supports
C0	Control cotton fabric (not dyed with natural extract)
C1	Dyed cotton fabric by exhaustion
C2	Dyed cotton fabric by sonication
C-EXH-3CA	Cotton fabric activated with citric acid mordant (3%) and dyed by exhaustion
C-EXH-3TA	Cotton fabric activated with tannic acid mordant (3%) and dyed by exhaustion
C-EXH-5TA	Cotton fabric activated with tannic acid mordant (5%) and dyed by exhaustion
C-EXH-5CA	Cotton fabric activated with citric acid mordant (5%) and dyed by exhaustion
C-EXH-3CS	Cotton fabric activated with copper sulphate mordant (3%) and dyed by exhaustion
C-EXH-5CS	Cotton fabric activated with copper sulphate mordant (5%) and dyed by exhaustion
C-US-3CA	Cotton fabric activated with citric acid mordant (3%) and dyed by sonication
C-US-3TA	Cotton fabric activated with tannic acid mordant (3%) and dyed by sonication
C-US-3CS	Cotton fabric activated with copper sulphate mordant (3%) and dyed by sonication
C-US-5TA	Cotton fabric activated with tannic acid mordant (5%) and dyed by sonication
C-US-5CA	Cotton fabric activated with citric acid mordant (5%) and dyed by sonication
C-US-5CS	Cotton fabric activated with copper sulphate mordant (5%) and dyed by sonication

Chromatic characteristics of dyed cotton samples

The CIELAB chromatic parameters (L*, a*, b*, C*, h values) of cellulosic fabrics samples dyed with *Paeonia officinalis* L. extract were acquired by using the Tools II Plus software of the Datacolor 110 LAV reflection spectrophotometer under standard illuminant D65. The colour characteristics were evaluated from the L* (luminosity), a* (redness), b* (yellowness), C* (chroma) and h (tone/hue) values. Comparison to control sample was done. The colour differences (ΔE) were calculated.

Attenuated total reflection FTIR analysis

Fourier Transform-infrared (FT-IR) measurements were carried out using an ALPHA FT-IR spectrometer (Bruker, Germany) with the combined QuickSnap™ sampling modules and ZnSe ATR (attenuated total reflection), with a resolution of 4 cm^{-1}. An average of 32 scans was recorded in the attenuated total reflection (ATR) mode.

Statistical analysis

Data were expressed as mean±standard deviation of two replicates. The differences between extraction variables were statistically compared by applying the Kruskall-Wallis one-way analysis of variance using Systat v. 12.0 (Kruskal, Wallis). Linear and nonlinear models were obtained between process variables and total anthocyanins content.

Results and Discussions

Optimal conditions for anthocyanins high recovery using conventional and ultrasound-assisted extraction

The extraction of anthocyanins from vegetal sources is of great importance for industrial application of natural colorants.

The conventional extraction of total anthocyanins (TA) from common peony petals was initially performed by maceration using two type of solvents (water, 70% ethanol), three values of solvent/solid ratio (48/1, 24/1 and 12/1) and three different temperatures (30ºC, 40ºC and 50ºC). Ethanol was selected based on its safety compared to that of other commonly used organic solvents, which may favour extraction of anthocyanins, but exhibit adverse effects.

The adequacy of different mathematical models was tested such as to identify the relationship between TA content and process variables. According to the sequential model sum of squares, the suggested model that best fits the data was the quadratic one. The 3-D response plots for both investigated extraction solvents are shown in Figure 1.

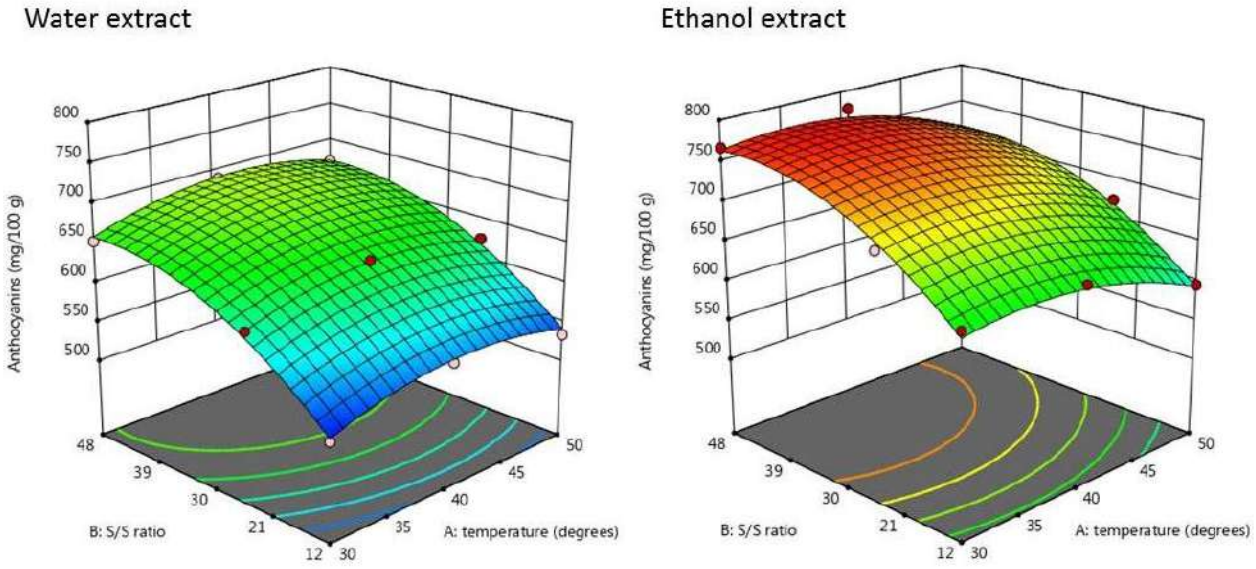

Figure 1. Response plots of the model for *Paeonia officinalis* anthocyanins content according to solvent/solid ratio and temperature; extraction by maceration.

According to the model summary statistics, the calculated coefficient of determination R^2 was 0.9876 showing a good fit model (p= 0.0001), while the adjusted and predicted R^2 were 0.9766 and 0.9766 indicating a close match between experimental and predicted values of the suggested model.

The determined TA content of common peony petals varied from 522.17 to 690.04 mg 100^{-1} DM in water extracts, and from 595.72 to 777.05 mg 100^{-1} DM in ethanol extracts. The maximum yield of TA extraction in both investigated solvents was obtained for the 48/1 solvent/solid ratio, at 40ºC, for 2 hs. Usually, temperature favours the extraction of molecules, but higher values might degrade heat sensitive compounds, such as anthocyanins. Our results indicated that tº > 40ºC did not lead to increased amounts of TA. The comparison of the efficiency of the two extraction solvents, under the same process parameters, showed that the best extracting one was 70% (V/V) aqueous ethanol. Ethanol improved extraction of anthocyanins compared to water (~ 13% average increase).

Based on these results, the optimization of the extraction conditions using pulsed ultrasound-assisted extraction (UAE) was performed using ethanol and further acidified ethanol, respectively at the following process parameters: solvent/solid ratio of 40/1 and 50/1, and three extraction time points (10, 20 and 30 min). The ultrasonic device variables were 50 and 70% amplitude, respectively. The measured temperature of the extracts after ultra-sonication varied between 24.0 and 32.4ºC.

According to the sequential model sum of squares, the suggested model that best fits the data was the Two Factor Interaction (2FI) one. The 3-D response plots for both investigated extraction solvents are shown in Figure 2.

A slight increase of the TA extractability in ethanol was found for higher solvent/solid ratio and extraction time. Lower ultrasonic amplitude (50%) determined higher TA mean values, compared to 70% amplitude, irrespective of solvent/solid ratio or extraction time, but was not statistically significant. The maximum TA content (874.98 mg $100\ g^{-1}$ DM) was determined in ultrasonic ethanol extracts, under the following conditions: 30 min, 70% amplitude, temperature < 23ºC during UAE extraction.

Usually, the addition of acids to the ethanol solution might favour the stabilization of anthocyanins in the form of coloured flavylium cation, so that ethanol acidified with 1 % citric acid, an organic acid of food grade, was hereby investigated. The results showed no improved yield of TA. Moreover, the differences between the efficiency of the two solvents as determined by Kruskall-Wallis one-way analysis of variance were statistically significant.

Mean values of TA content in ultrasonic ethanol extracts were higher than those of extracts obtained by maceration for 24 hours at 4ºC, but not statistically significant. Our study revealed higher TA content in ultrasonic ethanol extracts than that in extracts obtained by maceration, by 18%. Ultrasonication is preffered because maceration involves longer extraction times.

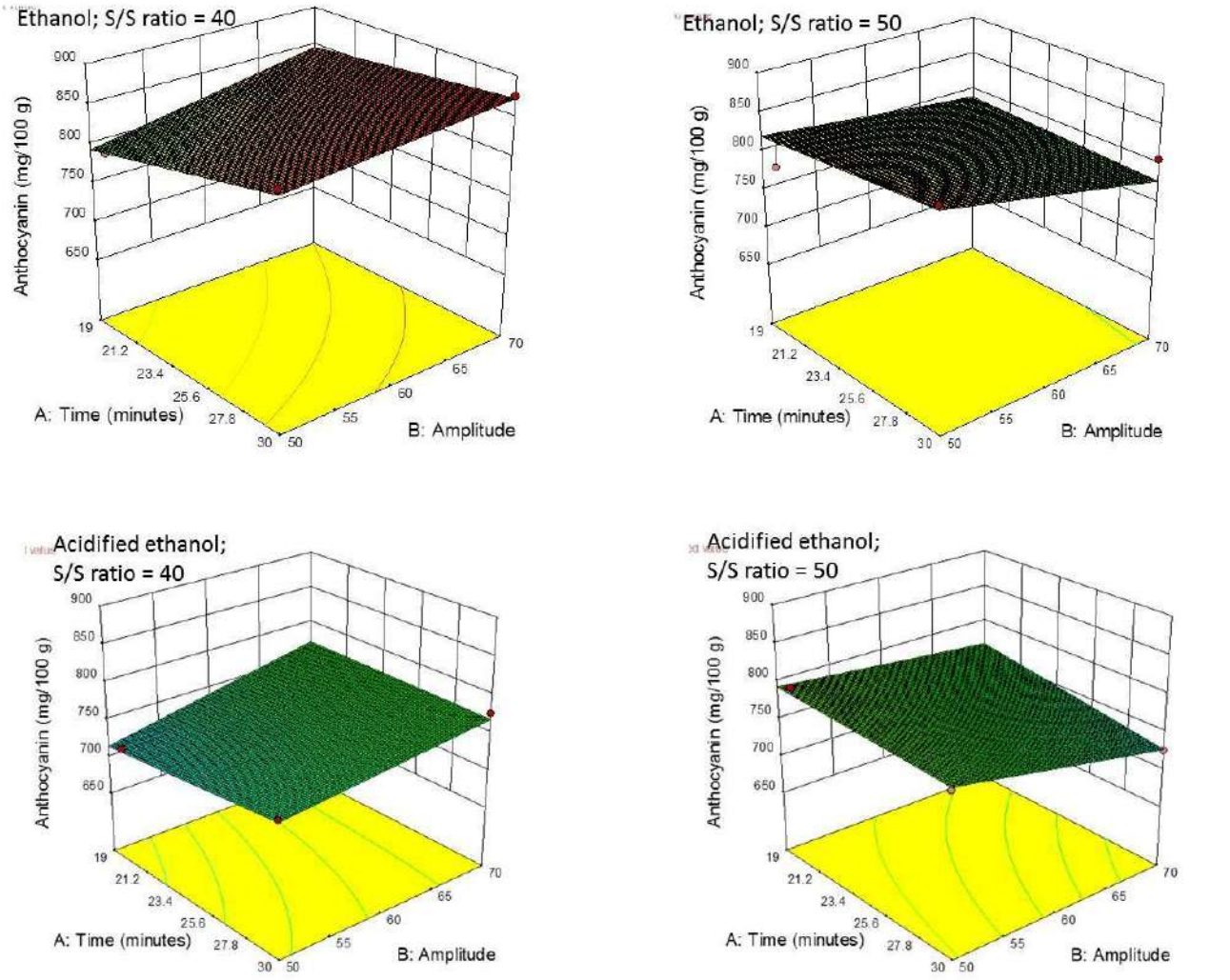

Figure 2. Response plots of the model for *Paeonia officinalis* anthocyanins content according to extraction time and ultrasonic amplitude, at different solvent/solid ratio; extraction by UAE.

To our knowledge, no studies regarding the optimization of conventional and UAE extraction of anthocyanins from common peony (*Paeonia officinalis* L.) have been published. Moreover, there are few studies reporting the TA content of herbaceous peony, but numerous papers describe the CIELAB chromatic components of fresh petals, based on the fact that colour is essential for botanical classification. A study regarding analysis of several Chinese and European species of herbaceous peony reported higher values (1.0483 mg 100mg^{-1} DW) for *Paeonia officinalis* ssp. *humilis* than those found by us (JIA, SHU, WANG, WANG et al, 2008). It might be related to different varieties and anthocyanins biosynthesis based on diverse climate areas and various experimental conditions, as well. In the mentioned study, extraction was done in methanol acidified with HCOOH and CF_3COOH at 4ºC for 24 hours, while TA composition was performed using HPLC. The authors identified six main anthocyanins in petals of 15 peony species, three of them (peonidin-3-*O*-glucoside-5-*O*-arabinoside, peonidin-3-*O*-glucoside-5-*O*-galactoside and cyanidin-3-*O*-glucoside-5-*O*-galactoside) being predominant in European species. Investigation on 48 cultivars of Zhongyuan tree peony revealed a TA content varying from 0 to 289 mg cyanidin 100 g^{-1} FW in 0.1% HCl acidified methanol extracts obtained by maceration at 4ºC for 24 hours (FERNANDES, CASAL, PEREIRA, SARAIVA, 2017).

Characterization of eco-dyed cellulosic substrates using peony crude extract

The ultrasonic ethanol extract of *Paeonia officinalis* L. which proved high recovery of total anthocyanins has been used for eco-dyeing of cotton fabrics by exhaustion and ultrasonication techniques, in the presence and absence of classic mordants (copper sulphate) and biomordants (citric and tannic acids) in concentration of 3 and 5%. At the 1:50 liquor ratio, the dyeing time and temperature by exhaustion procedure were 15 min and 80ºC.

The results of ATR-FTIR spectra of cotton samples, control and samples dyed with peony extract using two different procedures (conventional by exhaustion and ultrasonic method) in the presence and absence of (bio)mordants are shown in Figure 3.

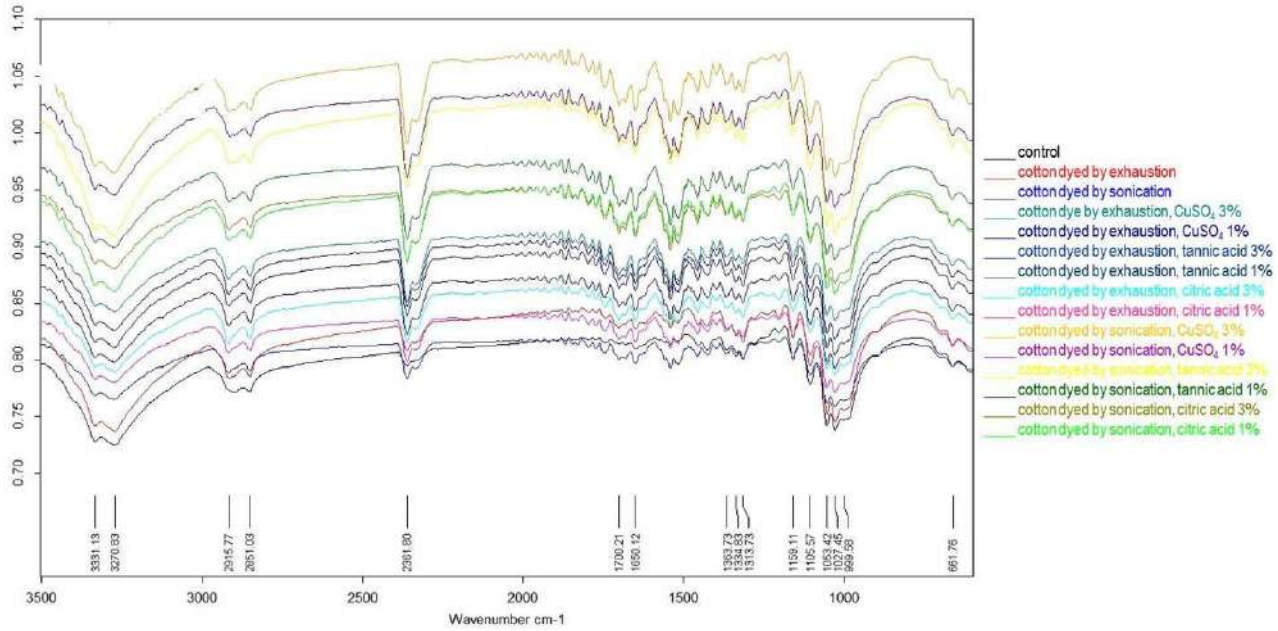

Figure 3. ATR-FTIR spectra of investigated cotton samples (conventional dyeing and ultrasonic dyeing with peony extract in the presence and absence of mordants).

The following characteristics can be noticed: the broad absorption band at 3330 cm^{-1} attributed to alcohol O-H stretching; bands at 2897 and 2851 cm^{-1} assigned to asymmetric and symmetric C-H bond stretching of cellulose; band at 1053 cm^{-1} attributed to alcohol C-O stretching. The strong band at 2361 cm^{-1} was attributed to carbon dioxide. Other peaks found only in dyed cotton samples were characteristic to peony extract indicating the presence of the natural dye, as shown in Figure 4. The results are in accordance with other reported ones (LEE, HWANG, KIM, 2009).

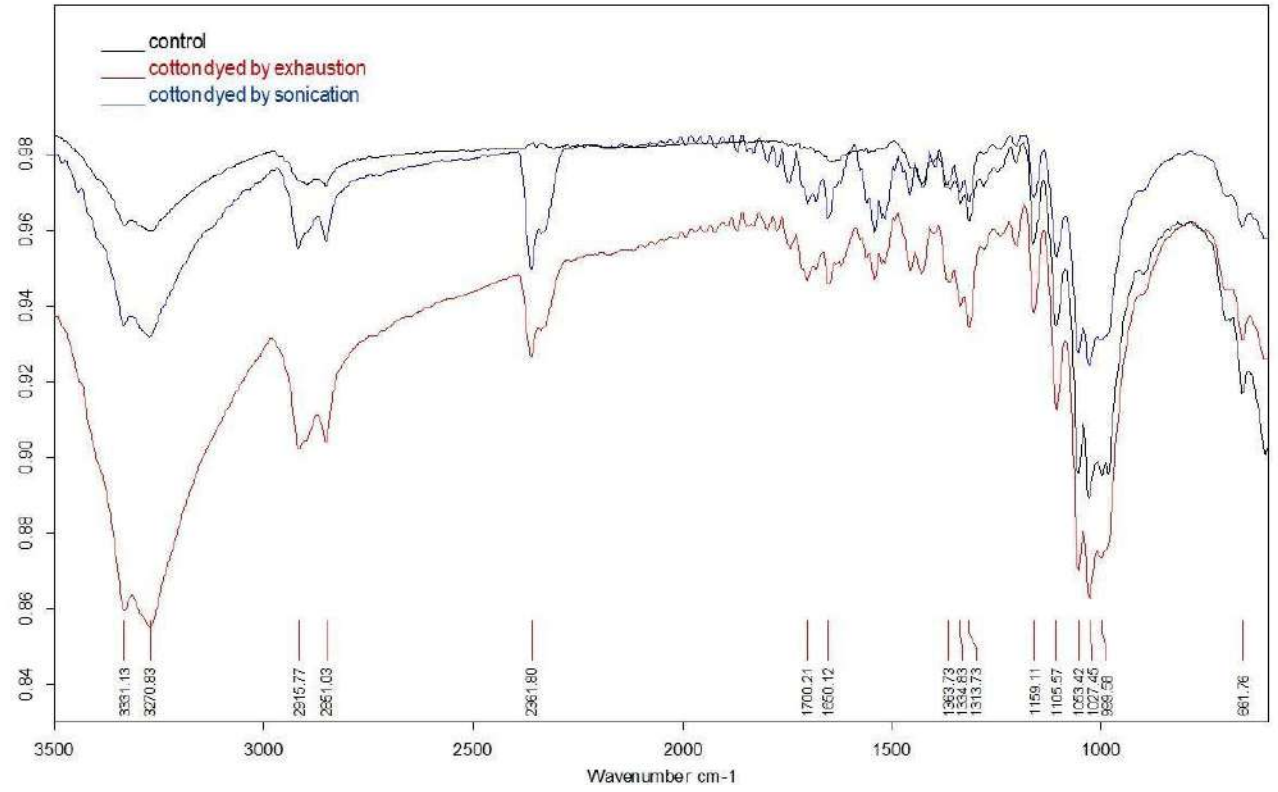

Figure 4. Comparative ATR-FTIR spectra of control and dyed cotton samples with peony extract without mordants.

The results of colour changes as measured by the CIELAB system for colorimetric data differences, ΔL*, Δa*, Δb*, ΔC*, ΔH* and colour differences ΔE* of dyed cellulosic supports with the obtained natural extract are presented in Table 3.

The values of luminosity (L*) and colour differences (ΔE*) changed in relation to the type of mordant used in the dyeing process.

The L* values increased in samples dyed with 3% citric acid (CA), using both standard and ultrasonication procedures. Low L* values indicating darkness were found in cotton dyed and assisted by copper sulphate (CS) and tannic acid (TA). The L* values of cotton dyed with *Paeonia officinalis* L. extract and assisted by mordants were found slightly lower than those reported by other authors (LEE, HWANG, KIM, 2009; SADI, FOISAL, NAHAR, 2016) probably due to the different anthocyanins composition of the investigated peony variety and different interaction with the mordants.

The highest a* (redness) values were obtained for samples dyed in the presence of CA as biomordant, while the highest b* values were observed for samples dyed using CS as classic mordant, compared to samples dyed without mordants (C1 and C2).

Large positive differences in chroma (ΔC*) were particularly found in cotton samples dyed by exhaustion with peony extract in the presence of high concentration of CA, CS and TA. Addition of 5% CA in the eco-dyeing process by exhaustion determined an increase of C* value (brighter) by more than 20 units showing an efficient type of cotton dyeing with peony extract. Low chroma values in samples dyed by ultrasonication showed the natural tone of the peony extract.

Increased ΔH* (hues) values were found in cotton samples dyed in the presence of CS, while decreased ones were found in samples dyed with CA, which recommend the use of CA as biomordant among the others for the stabilization of dyeing with peony anthocyanins extract.

Large colour differences (ΔE*) were observed for eco-dyed sample in the presence of high concentration of CS followed by samples dyed in the presence of CA and TA using the both type of dyeing (exhaustion and ultrasonication). These results indicate that biomordants did not significantly change the colour.

Table 3. The CIE colorimetric coordinates and colour changes in cotton samples dyed with *Paeonia officinalis* L. anthocyanins extract

Sample	ΔL*	Δa*	Δb*	ΔC*	ΔH*	ΔE*
C1	69.73	11.68	-5.06	13.01	337.09	-
C2	70.60	12.08	-6.13	13.55	333.12	-
C-EXH-3CA	4.67	11.84	0.06	11.14	4.13	13.97
C-EXH-3TA	-3.73	-2.94	4.46	-4.12	3.24	6.52
C-EXH-5TA	-7.23	14.48	3.14	13.18	6.76	16.48
C-EXH-5CA	-13.53	21.57	0.99	20.49	6.79	25.48
C-EXH-3CS	-10.25	1.95	20.84	6.78	19.80	23.30
C-EXH-5CS	-17.62	12.81	17.84	14.03	17.05	28.24
C-US-3CA	12.29	17.48	6.52	-8.27	5.48	15.79
C-US-3TA	2.35	-0.67	0.78	-0.91	0.48	2.56
C-US-3CS	1.05	-6.55	20.67	3.52	21.40	21.69
C-US-5TA	0.41	0.86	5.53	-0.16	5.59	5.61
C-US-5CA	0.93	7.12	1.65	6.40	3.54	7.37
C-US-5CS	-9.04	2.62	17.87	6.41	16.89	20.20

Conclusions

This study indicates the optimal extraction conditions of anthocyanins, as main dyes of herbaceous peony (*Paeonia officinalis* L.) flowers by using ethanol compared to water and acidified ethanol. Different mathematical models were tested in order to identify the relationship between total anthocyanins content and various process variables. The results showed higher mean values of total anthocyanins content in ultrasonic extracts, in particular at high solvent/solid ratio (50/1) and time (30 min), compared to those of extracts obtained by maceration for 24 hours at 4°C.

The obtained crude ethanol peony extract was used in cotton eco-dyeing *via* conventional procedure and ultrasonication, in the presence and absence of classic mordant (copper sulphate) and biomordants (tannic and citric acids). Successful dyeing was obtained according to ATR-FTIR analysis indicating characteristic peaks of peony extract in dyed cotton samples. The colour properties of dyed cellulosic substrates as determined by CIELAB system showed higher a* (redness) values and large positive differences in chroma (ΔC*) in samples dyed by exhaustion with peony extract in the presence of high concentration of citric acid. Large colour differences (ΔE*) were observed for eco-dyed sample in the presence of high concentration of copper sulphate followed by samples dyed in the presence of citric acid and tannic acid using the both type of dyeing (exhaustion and ultrasonication).

These results indicate an optimum ethanol ultrasound-assisted extraction of anthocyanins from *Paeonia officinalis* L. Moreover, the peony extract proved efficient in dyeing of cotton fabrics, in particular the biomordanted

ones, advancing directions to manufacturing textiles without negative impact on health and environment.

Conflict of Interest

The authors have no conflict of interest to declare.

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