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Natural Dyeing of Wool Using *Junglans regia* (Common Walnut) Leaf Extract

Naravno barvanje volne z ekstraktom iz listov Junglans regia (navadni oreh)

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Abstract

The main objective of the presented research was to study the possibility of using natural colourants obtained through the aqueous extraction of fresh leaves from the *Junglans regia* (*J. regia*), or common walnut tree, for the dyeing of wool yarn. A unique principle was explored by combining the phases of extraction and mordanting into one with the aim of shortening the dyeing procedure, while at the same time extracting more colouring components. Spectrophotometric studies revealed the significant impact of both mordant addition and dye-bath pH on the absorbance curve and thus on the colour and K/S values of the dyed samples. A meta-mordanting technique using ferrous sulphate produced a greater depth of shade at a wavelength of 400 nm, with respect to the concentration of mordant and the liquid ratio of the extraction. Finally, wool yarn dyed with pure leaf extracts exhibited a potent inhibiting activity against *Candida albicans* (*C. albicans*) with a moderate reduction rate of 59%, and an inhibited response against *Staphylococcus aureus* (*S. aureus*) with a low reduction rate of 38.6%.
Keywords: natural colourants, wool dyeing, walnut leaf extract, ferrous sulphate, colourimetry, antimicrobial activity

Izvleček

Namen predstavljene raziskave je bil proučiti možnost uporabe naravnih barvil, pridobljenih z vodno ekstrakcijo svežih listov iz drevesa Junglans regia (J. regia), navadnega oreha, za barvanje volnene preje. Raziskali smo edinstven pristop z združevanjem obeh faz, ekstrakcije in dodajanja anorganskih soli, v eno fazo, da bi skrajšali postopek barvanja in hkrati ekstrahiranja večje količine barvila za barvanje. Spektrofotometrična študija je pokazala pomemben vpliv uporabljene anorganske soli – čimže in pH barvalne kopeli na absorpcijsko krivuljo ter posledično na barvo in K/S vrednosti obarvanih vzorcev. Tehnika dodajanja železovega sulfata med barvanjem daje večjo globino obarvanja pri valovni dolžini 400 nm, to je odvisno od koncentracije oksida in kopelnega razmerja ekstrakcije. Prav tako smo dosegli zmerno protimikrobno delovanje obarvane volne proti glivičnemu sevu Candida albicans (C. albicans), z 59-odstotno stopnjo zmanjšanja in nizko stopnjo delovanja proti bakteriji Staphylococcus aureus (S. aureus) z 38,6-odstotno stopnjo zmanjšanja.
Ključne besede: naravna barvila, barvanje volne, ekstrakt orehovih listov, železov sulfat, barvna metrika, protimikrobno delovanje

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1 Introduction

Eco-friendly dyeing could be achieved through various approaches, i.e. using biodegradable auxiliaries, optimising the amount of chemicals, replacing toxic chemicals with eco-friendlier chemicals, employing natural colouring compounds, etc. [1–3]. This paper focuses on dyeing with natural colouring compounds that are derived from different parts of plants, such as flowers, leaves, bark, roots and fruits, using various extraction technique, and that are believed to be ecologically-friendly with fewer negative effects on the organism on account of their low toxicity and non-carcinogenic nature. Moreover, natural colourants are biodegradable and thus do not cause pollution and wastewater problems. On the other hand, they provide a lesser amount of colouring component and inferior fastness properties, resulting in higher production costs compared with synthetic dyes [1]. Textiles dyed with natural dyes could therefore be suitable as niche products of high-added value for special markets.

Although known for a long time for dyeing and medical purposes, the structure and protective properties of natural colourants were not identified until recent decades [4]. Different papers have reported the selection of a variety of plants/parts of plants, i.e. henna leaves [5], lady's bedstraw root and big nettle leaves [6], turmeric rhizomes, harda fruits, safflower petals and barberry roots [1], madder roots and onion peel [2], purple sweet potato [7], weld [8], etc. for the natural dyeing of various natural fibres, and the optimisation of different exhaustion and dyeing procedures using chemically-different mordants in order to achieve an extensive palette of colour shades, and good to excellent colour-fastness. In this study, common walnut leaves were chosen as the source for a plant-based reddish-brown natural dye. The leaves are a promising dye source because of their easy availability and abundant nature [9]. Moreover, the removal of leaves is less harmful to trees compared with the stripping of bark. *J. regia* (common walnut) is a deciduous tree of the *Junglandaceae* family native to south-eastern Europe [10]. It reaches a height of up to 25–35 m and a diameter of up to 2 m. The water extracted from walnut leaves is reported to exhibit powerful antioxidative [10] and antimicrobial properties [1, 2] owing to the presence of large amounts of phenolic compounds, such as naphtoquinones and flavonoids, depending on the agricultural, geographical, and climatic conditions. From among the naphtoquinones, the juglone (5-hydroxyl-1,4-naphthoquinone; Figure 1) is of great interest due to its chemical reactivity. Because of its tendency to create dark orange-brown stains, juglone has been used traditionally as a natural dye for clothes and inks, and as a colouring agent for foods and cosmetics. It is also a well-known and widely used substance in folk medicine for the treatment of skin inflammations, hyperhydrosis, ulcers, venous insufficiency, and haemorrhoidal symptomatology, and for its proven antiseptic, anti-diuretic, antistringent and sedative properties [10].

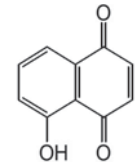

Figure 1: Chemical structure of 5-hydroxyl-1,4-naphthoquinone

Only a few natural dyes are substantive to fibres; all others require inorganic oxides or metallic salts, such as ferric sulphate, aluminium sulphate, potassium dichromate, stannous chloride and copper sulphate, known as mordants, which form a complex with a dye *in situ* within the wool fibre, resulting in a dramatic improvement in both the fastnesses of the dyeing to light and washing [11]. Three methods for mordant application are commonly used: pre-mordanting, meta-mordanting and post-mordanting. The method used depends on whether the mordant is applied before, during or after dyeing.

A unique principle was explored during the presented work by combining the phases of extraction and mordanting into one with the aim of shortening the dyeing procedure, while at the same time extracting more colouring compounds. Thus, ferrous sulphate, an eco-friendlier mordant (compared with the metal salts listed above) was added at the beginning of the extraction procedure to form a complex between the colourant and a metallic ion, as in the case of synthetic (pre-metallised) metal-complex dyes. For comparison purposes, standard dyeing and meta-mordanting using various amounts of metallic salts were also carried out. Thus, different factors affecting exhaustion ability and fastness properties were investigated to demonstrate commercial viability and to meet demands for eco-friendliness. The

colorimetric parameters (e.g. CIE colour values, colour differences and colour strength or K/S value) of the dyed samples were identified, as we were potential antimicrobial properties against common human pathogens, such as *C. albicans* and *S. aureus*, in accordance with the ASTM specification.

2 Experimental

2.1 Materials

Experiments were conducted on a rough, yellowish-coloured wool yarn (L^* = 75.10, C^* = 16.48, h = 90.11, and whiteness according to CIE –42.5), made from Slovenian sheep's wool by the company Soven (Selnica ob Dravi, Slovenia) for hand knitting, with a tow length of 73.6 mm, fineness of 82 × 2 tex and a fat content of 1%. The source yarn was pre-washed at 40 °C for 20 minutes using a neutral non-ionic washing agent in order to remove natural grease and potential additives. It was then rinsed in warm and cold water, and dried at a temperature of 60–70 °C. Ferrous sulphate ($FeSO_4 \times 7H_2O$) and other chemicals, such as sodium carbonate (Na_2CO_3) and acetic acid (CH_3COOH), were analytically graded reagents obtained from Sigma Aldrich.

2.2 Extraction of dye

J. regia leaves were collected during the summer (June 2016) in central Slovenia at an elevation of 240 m above sea level and a high thermal amplitude. The adult walnut trees selected for this research were not sprayed with pesticides, dunged or otherwise agronomically cultivated. The trees were also without traces of disease or pests.

Fresh leaves were separated from the stalks and chopped into small pieces. The extraction was carried out in deionised water using three liquor ratios, i.e. 1:10, 1:30 and 1:50 (1 g of plant material to 10, 30 or 50 mL of deionised water), with the aim of obtaining different concentrations of extracted colourants. The extraction was accomplished without mordanting (classical extraction) and with the addition of 2 g/L $FeSO_4 \times 7H_2O$ at the beginning of the extraction procedure.

The extraction procedure was carried out in a covered glass beaker at boiling temperature for 60 minutes. Afterwards, the extraction mixture (e.g. the extract and plant material) was kept at room temperature for approximately 18 hours and then filtered for further use.

After the extraction was completed, the pH value of individual extracts was measured (pH_E) using a MA 235 pH meter (Mettler Toledo) in accordance with the ISO 10523 standard, and then adjusted to a value of 3, 5 or 8 by adding CH_3COOH or Na_2CO_3 for spectrophotometric analysis. The extracts were characterised by measuring absorbance through the entire UV/Vis spectrum, from a wavelength of 250 nm up to 700 nm, using a 10 mm quartz cuvette on a Cary 50 spectrophotometer (Varian).

2.3 Dyeing procedures

A total of 10 g of wool yarn was dyed according to two dyeing procedures, i.e. standard dyeing and meta-mordanting, at a temperature of 98 °C and a liquor ratio of 1:20, using a Turby laboratory device (W. Mathis) with a medium bath circulation.

Standard dyeing was carried out using two extraction baths, i.e. pure walnut leaf extract and an extract with 2 g/L of ferrous mordant that was applied at the beginning of the extraction procedure. Three different initial concentrations of the extracted colourants were used, as the liquor ratios of extraction (LR_E) were 1:10, 1:30, or 1:50. The dyeing process was started at 24 °C when the wool yarn was added to the extracted bath, and the pH adjusted to 3 or 5. The temperature of the dye-bath was raised to 98 °C at a heating rate of 2 °C/minute. That temperature was maintained for 60 minutes and then reduced to 70 °C (at a rate of 2 °C/min). The dyed yarn was rinsed in warm and then cold water, and dried at room temperature.

In the case of meta-mordanting, dyeing was started at 24 °C by placing the wool yarn in a pure extracted bath that was obtained from the same quantity of fresh leaves (30 g) against a different volume of deionised water; LR_E was 1:10, 1:30 and 1:50. The temperature of the dye-bath was then gradually raised to 98 °C at a heating-rate of 2 °C/minute. Dyeing continued for 15 minutes. The dye-bath was then cooled to 70 °C (at a rate of 2 °C/min), when $FeSO_4 \times 7H_2O$ (0.5, 1 or 2 g/L) was added. The temperature of the dye-bath was again raised to 98 °C at a heating rate of 2 °C/minute. That temperature was maintained for 45 minutes and then reduced to 70 °C (at a rate of 2 °C/min). The dyed yarn was rinsed in warm and then cold water, and dried at room temperature, similar to the standard dyeing procedure.

Absorbance was measured on-line throughout the entire dyeing process using a Cary 50 spectrophotometer (Varian) with a measuring probe of 0.2 mm

optical length at a wavelength of 400 nm. In addition, the dye exhaustion rate was calculated using the following equation:

$$ER = \frac{A_0 - A_t}{A_0} \times 100\ (\%) \quad (1),$$

where ER is the dye exhaustion rate expressed as a percentage, A_0 is the initial absorbance and A_t is the absorbance over a fixed period.

2.4 Colour measurement

The dyed samples were colourimetrically evaluated according to the CIE colour system using a two-ray SF 600+ spectrophotometer (Datacolor) with an Ulbricht sphere and a measuring geometry of d/8°, within the spectral range of 400–700 nm wavelengths. The source of light was a halogen lamp with xenon lightning.

CIE differences in lightness (dL^*) and total colour differences (dE^*) between various dyed samples (original samples were dyed using the standard dyeing procedure) were calculated (equation 2) from the coordinate differences in all three directions of the colour space, i.e. lightness L^*, red/green axis a^* and yellow/blue axis b^*. C^* is the abbreviation for chroma and h for hue.

$$dE^*_{ab} = \sqrt{(dL^*)^2 + (da^*)^2 + (db^*)^2} \quad (2)$$

The colour strength of individual samples (K/S) was calculated from the reflectance values at 400 nm for each dyeing using the Kubelka-Munk equation:

$$K/S = \frac{(1 - R)^2}{2R} \Big) \quad (3),$$

where K is the absorption coefficient, S is the light-scattering coefficient and R is the decimal fraction of the dyed sample's reflectance.

2.5 Fastness testing

The colour fastnesses of the samples to washing was tested according to the EN ISO 20105-C01 Standard; Test 1: washing at 40 °C. Colour-fading and colour-leaching from dyed samples on two control strips made from cellulose and wool were visually evaluated using a normalised grey scale (on a scale of 1–5, where 1 = poor and 5 = excellent). The colour fastnesses of samples to light was tested using artificial illumination from a xenon arc light according to the EN ISO 105-B02 Standard and visually estimated using a standardised blue scale (on a scale of 1–8, where 1 = poor and 8 = excellent).

2.6 Antimicrobial screening test

ASTM Designation: The E 2149-01 Standard test method was used to assess the non-leachable antimicrobial activity (both bacterial and fungal) of the dyed wool yarns under dynamic contact conditions (24-hour contact time at ambient temperature). The analysis was performed by a certified laboratory. A gram-positive bacterium *S. aureus* and fungus *C. albicans* were selected. The reduction of the microorganisms by dyed yarn was calculated using equation 4:

$$R = \frac{(B - A)}{B} \times 100\ (\%) \quad (4),$$

where R is the reduction of the microbial population expressed as a percentage, A is the number of bacteria colonies (CFU/mL) for the flask containing the treated sample after 1 hour contact time and B is the number of bacteria colonies (CFU/mL) for the flask to determine A before the addition of the treated sample (time 0).

3 Results and discussion

With the aim of developing textiles of high added-value for special applications, the presented research work focused on the search for a mordant concentration, a mordant application technique and the quantity of fresh leaves per volume of water for extraction, as well as the optimal application conditions for obtaining natural dyes in a wide colour palette, with high colour-strength and outstanding fastness properties. The antibacterial and antifungal properties of dyed samples were also investigated. The obtained results and the relevant discussion are presented below.

3.1 UV/Vis analysis of the aqueous walnut leaf extract

Two extracted baths at LR_E 1:50, i.e. one bath without and one bath with 2 g/L of $FeSO_4 \cdot 7H_2O$, which was employed at the beginning of the extraction procedure, and at three pH values (3, 5, and 8), were characterised using spectroscopic measurement in order to study the effect of mordant application and the acidic or alkaline pH on colour hue. A turbid solution was formed during the extraction procedure. Part of the absorbance was thus probably due

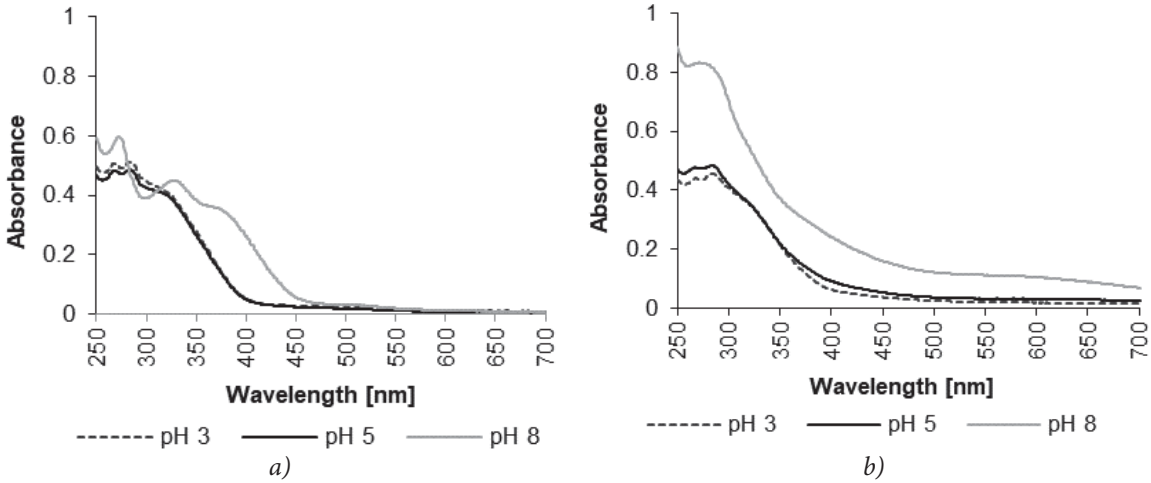

Figure 2: Influence of pH and mordant on the absorbance of walnut leaf extract, diluted 25x: a) pure extract mordant excluded; b) extract with $FeSO_4 \cdot 7H_2O$

to the presence of insoluble substances, which caused a problem in the spectrophotometric analysis, irrespective of the preceding filtration.

It is evident from Figure 2a that aqueous extraction at a boiling temperature removed various colouring compounds from the walnut leaves, thus influencing the appearances of two absorption maximums in the UV region at wavelengths of 268 nm and 283 nm. Pure walnut leaf extract (mordant excluded) had a pH value of 5 by itself, probably on account of various hydroxycinnamic and chlorogenic acids present in fresh leaves, which have been seasonally deviated and identified by different authors, using various skills [12, 13]. When pH is changed from acidic (pH values of 3 and 5) to alkaline (pH value of 8), the absorbance curves are shifted towards higher wavelengths; colour thus changes. This could be due to (i) the higher solubility of the extracted colourants at higher pH values, (ii) the deacidification of water-colour pigment and/or (ii) the fact that the structure of juglone may be represented in more potentially resonant forms, thus allowing for greater electronic distribution. Furthermore, the application of the selected mordant (Figure 2b) results in a different colour. The addition of Fe-salt to the extract yielded the corresponding Fe-complex of the extracted hydroxynaphthoquinone components, resulting in increased absorbance [14]. According to the analogy of 1-hydroxyantraquinone derivatives, juglone contains, as a basic unit, a six-membered chelate ring in which the metal ion is coordinated with the oxygen atom of the 1-hydroxy group and the quinone oxygen atom (Figure 3). Mordanting causes the colour of a dye to undergo an appreciable bathochromic shift because the donor properties of the hydroxyl group and the acceptor properties of the carbonyl group are both enhanced. Also, the ionisation of a hydroxynaphthoquinone dye by varying pH results in an irreversible bathochromic shift, as the oxide group is a more powerful electron donor than the hydroxyl group.

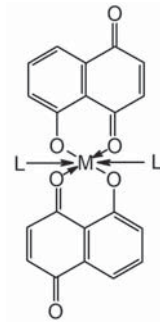

Figure 3: Scheme of coordination complex between juglone and metal ion; M = metal, L = ligand

3.2 Dye sorption

In order to observe the sorption degree of the extracted colourants onto wool fibres and the influence of metallic salt on exhaustion, a standard dyeing procedure was carried out using two extracted baths at LR_E 1:50, follows: one dye-bath mordant excluded and one mordant included at two pH values (3 or 5), as wool fibres are commonly dyed at acidic pH values. Therefore, dye-bath absorbance

was followed by on-line UV/Vis spectrophotometry throughout the entire dyeing process at a wavelength of 400 nm (extracted colourants have badly defined maximums within a visible region of the spectrum), while the exhaustion rate was calculated according to equation 1. Also, the pH of the dye-baths was measured on-line by means of a temperature-resistant electrode. The selected results of dye exhaustion rate *versus* dyeing time/temperature are graphically presented in Figure 4. Exhaustion curves define the distribution of the dye between the dye-bath and wool fibres during the dyeing process, and indicate that both the dye adsorption on the wool fibres' surfaces and the dye-diffusion into the fibres depend on the time and temperature, and on the pH of the dye-baths and mordant application method.

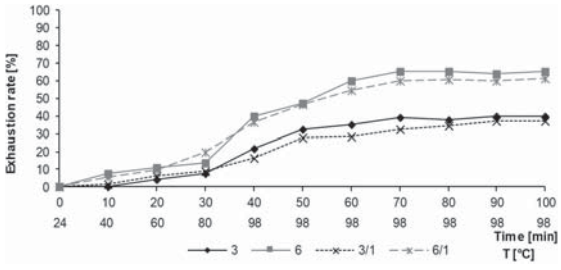

Figure 4: Selected exhaustion curves of representative dyeing using LR_E 1:50; 3 dyed at pH 5 mordant excluded, 6 dyed at pH 5 using $FeSO_4$, 3/1 dyed at pH 3 mordant excluded, and 6/1 dyed at pH 3 using $FeSO_4$

Absorbance was also measured during the meta-mordanting procedure, but the exhaustion rate could not be calculated. When metallic salt was applied during dyeing at 70 °C, enormous decreases in pH were observed, as well as major increases in absorbance at 400 nm. Thus, the initial absorbance (A_0) required for the exhaustion calculation (Equation 1) could not be the same for the whole process and is thus irregular.

The obtained results in Figure 4 demonstrate that the presence of mordant in the dye-bath could influence the dyeing behaviour of wool yarn, depending on the time and dyeing temperature, and on the pH of the dye-bath. The exhaustion rate increased very slowly for the representative trials during the first division of the dyeing process (first 28 minutes) when the dye-baths were heated, implying that the temperature required to start the exhaustion of natural colourants extracted from walnut leaves must be between 80 °C and 98 °C. From amongst the on-line absorbance measurements, the best sorption at the end of the individual dyeing process was achieved using a dye-bath containing ferrous sulphate pH 5, which is comparable with reported results [15, 16]. The exhaustion rate of the colourants from the dye-bath's mordant included after 100 minutes of dyeing at pH 5 was 65.3%, followed by an exhaustion rate under pH 3 dyeing of 61.4%. The obtained results were exceedingly low compared with the exhaustion of synthetic metal-complex dyestuffs that can attain values in excess of 90% [17]. According to literature, the situation in mordant dyeing with natural colourants is more complex than when synthetic metal-complex dyes are used [18]. Besides the change in the ionisation of the wool, the pH-dependent sorption of the Fe-ions on wool and the complex stability between the extracted colourants and the mordant must be considered.

Moreover, the exhaustion curves show gentler slopes at both pH values during dyeing using pure leaf-extract (mordant excluded) on account of the lower affinity of the colouring components to the substrate compared with the metal-colourant-complex formation. Nevertheless, all results illustrated in Figure 4 still exhibit some increases in exhaustion rate at the end of the dyeing period, and thus indicate the possibility of exhausting higher amounts of dyestuff-forming substances by prolonged dyeing time at boiling.

It can be concluded from the dye-uptake results of the representative experiments that dyeing conducted at a lower pH value (pH 3) leads to a lower exhaustion rate, and consequently to lighter dyeing. For this reason, all other dyeing experiments (standard dyeing and meta-mordanting) were carried out at pH 5.

3.3 Colour evaluation

CIE colour values and colour differences

With the aim of illustrating the wide-range of colours achieved, the CIE colour coordinates of samples dyed with walnut leaf extract using three different liquid ratios of extraction (1:10, 1:30 or 1:50), according to two dyeing procedures (standard dyeing and meta-mordanting), are given in Table 1. In total, 15 samples were dyed and colourimetrically evaluated. Moreover, the differences in lightness (dL^*) and the total colour differences (dE^*) were calculated between samples 1, 2 and 3 (standard samples), and all other samples at the same LR_E, using equation 3, in order to evaluate the effects of the

Table 1: CIE colour values and colour differences for dyed wool yarn using different dye-baths and dyeing procedures

Procedure	Sample number	LR_E	Mordant $FeSO_4$ c (g/L)	L^*	a^*	b^*	C^*	h	dL^*	dE^*
Standard dyeing (dye-bath mordant excluded) Standard dyeing (dye-bath mordant included)	**1**	1:10		43.37	11.96	22.38	25.37	61.88		
	2	1:30		47.42	10.88	20.96	23.62	62.57		
	3	1:50	2	55.95	9.98	20.31	22.63	63.83		
	4	1:10	2	35.36	0.38	12.50	12.51	88.25	–8.00	17.20
	5	1:30	2	40.27	0.73	12.06	12.06	86.54	–7.15	15.28
	6	1:50		45.72	0.79	13.15	13.18	86.55	–10.23	15.50
Meta-mordanting	**7**	1:10	0.5	24.10	4.47	11.96	12.77	69.53	–19.27	23.16
	8	1:10	1	22.61	3.83	10.49	11.17	69.93	–20.76	25.27
	9	1:10	2	20.22	3.11	8.85	9.38	70.62	–23.15	28.24
	10	1:30	0.5	27.83	3.59	11.70	12.23	72.96	–19.59	22.86
	11	1:30	1	24.25	2.85	10.08	10.48	74.23	–23.17	26.82
	12	1:30	2	22.89	2.43	9.55	9.85	75.70	–24.53	28.34
	13	1:50	0.5	32.92	2.78	11.70	12.03	76.63	–23.03	25.97
	14	1:50	1	28.63	2.35	10.27	10.53	77.10	–27.32	30.44
	15	1:50	2	27.70	2.24	9.88	10.13	77.21	–28.25	31.44

mordant, as well as the dyeing procedure, on the colour change. Because the preliminary sorption experiments showed better dye-bath exhaustion under slightly acidic conditions (Figure 4), all dyeing trials were carried out at pH 5.

It can be concluded from Table 1 that yarn dyed with walnut leaf extract showed some decrease in L^* and C^* values when metallic salt was added. That decrease became more apparent as the liquor ratio of extraction (LR_E) decreased and when the concentration of mordant increased (samples 7–15). Also, the application of mordant caused changes in the shades of samples. Generally, $FeSO_4$ mordant gives bluer and less red hues, when compared to the original non-mordanted samples dyed using the standard dyeing procedure (samples 1–3). The meta-mordanting technique using iron sulphate has a major influence on CIE colour values (samples 7–15) compared with the standard dyeing procedure's mordant excluded (samples 1–3). Total colour differences were between 22.86 and 31.44, while differences in lightness were between –19.27 and –28.25, as could already be expected on the basis of reported colouration results from different authors dealing with various natural colourants [9, 16, 18, 19]. Notable differences were also observed between samples dyed in dye-baths, where iron salt was added at the beginning of the extraction procedure (samples 4–6), and samples dyed using the meta-mordanting technique (samples 7–15); dE^* ranging from 12 to 22.26 and dL^* from 11.26 to 22.03.

Colour strength and fastness properties

An important aspect when selecting plant material for dyeing is the maximum colour depth that can be achieved. The extent and nature of this colour strength is illustrated by the K/S value versus wavelength plot, as shown in Figures 5 and 6.

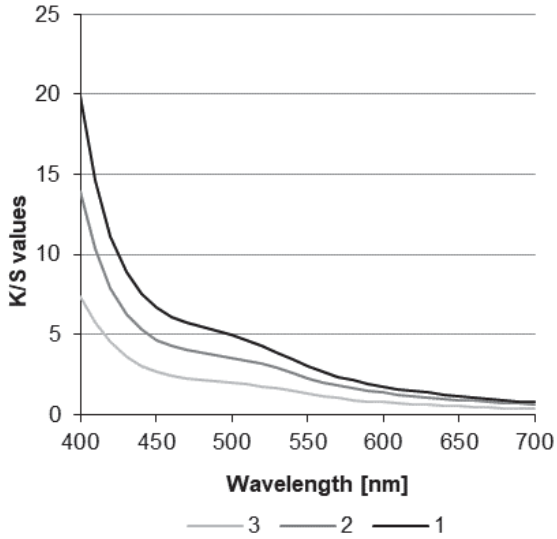

Figure 5: Influence of extraction liquor ratio on the K/S values of samples dyed using pure leaf extract; 1 – LR_E 1:10, 2 – LR_E 1:30, 3 – LR_E 1:50

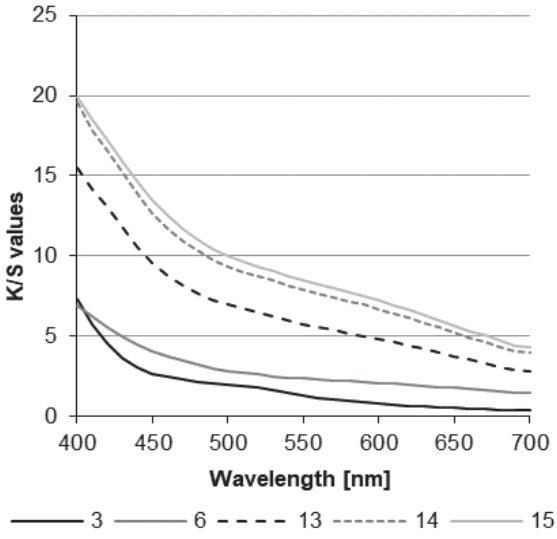

Figure 6: Influence of mordant and dyeing procedure on the K/S values of samples dyed using representative dyeing methods; LR_E 1:50; standard dyeing: 3 – without mordant, 6 – 2 g/L of $FeSO_4$; meta-mordanting: 13 – 0.5 g/L $FeSO_4$, 14 – 1 g/L $FeSO_4$, 15 – 2 g/L $FeSO_4$

The *K/S* values illustrated in Figure 5 indicate a decrease in the colour strength of dyed samples when the liquor ratio of walnut leaf extract increased, as could be expected from the absorbance measurement. However, the *K/S* value versus wavelength curves do not reflect the finding characteristic for synthetic dyes, where *K/S* measured at maximum wavelength increases equally with an increases in the dye concentration, resulting in a direct correlation between the colour strength and the dye absorbance/concentration. The addition of iron salt during dyeing leads to a major increase in the *K/S* values throughout the entire visible spectrum, compared with the original (non-mordanted) representative sample 3 (Figure 6). On the other hand, standard dyeing, using dye-baths' mordant included, caused a minor improvement in the colour strength.

Table 2 shows the influence of extraction liquor ratios, dyeing procedures, and the type and concentration of the mordant on the *K/S* values measured at a wavelength of 400 nm, and on the wash and light fastness properties of the resulting natural dyes.

It is clear from Table 2 that meta-mordanting under a given set of experimental conditions leads to the significant enhancement of colour strength, producing dyeing with better fastness properties than those dyed according to the standard procedure. Metal ion is well-known for its ability to form a coordinated complex, as the coordination number of iron is 6. Some coordination sites remained unoccupied when it interacted with the fibre. Thus, the amino groups on wool fibre occupied these sites (coordination bonding). Such a strong coordination tendency enhanced the interaction between the fibre and the dye, resulting in higher dye exhaustion compared with mordant-excluded standard dyeing (hydrogen bonding between -OH groups of dyes and carboxyl groups of wool fibre).

3.4 Determination of antimicrobial activities

Generally, textiles are carriers of microorganisms, such as pathogenic bacteria, odour-generating bacteria and moulds, as they provide larger surface areas and adsorb the moisture required for microbial growth and multiplication, leading to dermal infections, allergic responses, product deterioration, etc. [1, 2]. Because of the presence of large amounts of phenolic compounds in walnut leaf extract [10, 12], which are classified as active antimicrobial compounds, we presumed that textile materials dyed using such an extract could enhance the antimicrobial abilities of textiles. The presented research was therefore undertaken to determine the antimicrobial effectiveness of selected non-mordanted original dyed yarn (sample 3), as well as mordanted samples dyed

Table 2: K/S values at 400 nm and colour fastness to washing and light for dyed wool yarn using different dye-baths and dyeing procedures

Procedure	Sample number	LR_E	Mordant $FeSO_4$ c (g/L)	K/S	Wash fastness W/S/C[a)]	Light fastness
Standard dyeing (pure dye-bath; original shade) Standard dyeing (dye-bath mordant included)	1	1:10		19.90	3/1–2/3–4	3
	2	1:30		13.91	3/1–2/3–4	3–4
	3	1:50		7.35	3–4/2/4	3–4
	4	1:10	2	18.29	3–4/3/4	2–3
	5	1:30	2	14.45	3–4/3/4	2–3
	6	1:50	2	6.91	4/3–4/4	3–4
Meta-mordanting	7	1:10	0.5	30.65	3–4/3/3–4	3
	8	1:10	1	31.69	3–4/3/3–4	3
	9	1:30	2	32.61	3–4/3–4/4	2
	10	1:30	0.5	23.58	3–4/3–4/3–4	3–4
	11	1:30	1	26.35	3–4/3–4/4	3–4
	12	1:50	2	27.81	3–4/4/4	2–3
	13	1:50	0.5	15.49	3–4/3/3–4	3
	14	1:50	1	19.56	3–4/3–4/3–4	3
	15	1:10	2	19.93	3–4/4/4	3–4

[a)] W/S/C – staining on wool/change in colour of sample/staining on cotton

using two dyeing procedures, i.e. standard dyeing with a dye-bath mordant included (samples 4 and 6) and meta-mordanting (samples 7–15), with the aim of studying the influence of concentration of metallic salt and dyeing procedures on the growth and metabolism of two pathogens, *S. aureus* and *C. albicans*. The results are expressed as the relative reduction of inoculated microbes and are shown in Figure 7.

It is evident from Figure 7 that the yarn dyed with pure walnut leaf extract used as a model sample (sample 3) exhibited a potent inhibiting activity against C. albicans with a moderate reduction rate of 59%, and a low inhibited response against S. aureus, with a reduction rate of 38.6%. The major drawbacks of both antimicrobial strains' functions were seen when iron salt was applied, regardless of the dyeing procedure, the nature of the used microorganism and salt concentration during meta-mordanting: the higher the salt concentration, the lower the antibacterial activities of the textiles. In the case of mordant dyeing, a strong complex is formed between the colourant and the wool fibre, resulting in lower activity against the selected pathogens, although the exhaustion rate is higher compared to dyeing without a mordant.

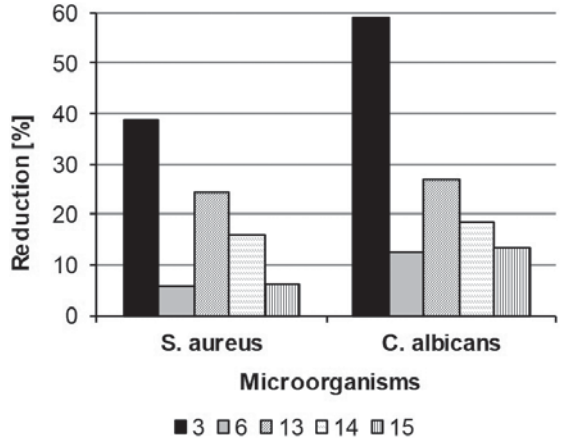

Figure 7: Percentage reduction of growth of two pathogenic strains (S. aureus and C. albicans) on the representative dyed yarns; LR_E 1:50; standard dyeing: 3 – without mordant, 6 – 2 g/L of $FeSO_4$; meta-mordanting: 13 – 0.5 g/L $FeSO_4$, 14 – 1 g/L $FeSO_4$, 15 – 2 g/L $FeSO_4$

4 Conclusion

Fresh leaves from the *J. regia* tree (common walnut) represent an inexpensive, ecologically-friendly raw

material that can be used for the extraction of reddish-brown natural colouring compounds. In this paper, a pure aqueous extract and an extract with $FeSO_4$ mordant included were used to dye wool yarn with the aim of achieving a wide palette of colour shades and good to excellent colour fastness, depending on the pH of the extract, the liquor ratio of the extraction, the type and amount of the applied mordant, and the type of dyeing procedure. Spectrophotometric studies revealed the significant impact of both the addition of a mordant and the pH of the dye-bath on the absorbance curve and thus on the hue and K/S values of the dyed samples. It can be concluded from a calorimetrical evaluation of the dyed samples that the application of $FeSO_4$ mordant gives bluer and less red hues compared to the original non-mordanted samples, regardless of the dyeing procedure. Generally, the meta-mordanting technique using iron salt gave a greater depth of shade than standard dyeing at a wavelength of 400 nm, regardless of the concentration of the mordant and the liquid ratio of the extraction. Finally, antimicrobial tests demonstrated a moderate antifungal activity of dyed wool samples against *C. albicans,* and a low antibacterial activity against *S. aureus*, as $FeSO_4$ reduced the inhibitory response against both pathogens.

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