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Coloration Technology

FEATURE ARTICLE

# Biomordanting willow bark dye on cellulosic materials

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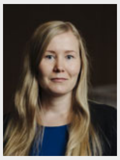
**Abstract**

A semi-quantitative study of willow bark dye adsorption on two different cellulose materials using biomordants was carried out. The studied celluloses were microcrystalline cellulose (MCC) AaltoCell and regenerated Ioncell-F (IC) fibres. The dye was a hot water extract of willow bark and the adsorption to cellulose was carried out using carboxylic acid-containing biomordants, namely, oxalic acid, citric acid and tannic acid. Alum was employed as the reference mordant. A semi-quantitative estimation of the dye uptake was conducted using high-performance liquid chromatography equipped with a diode array detector and also by visual inspection, as well as an evaluation of the coloration using CIELab parameters. The mechanism of the dye adsorption on the cellulose surfaces was studied via Fourier Transform–infrared spectroscopy. According to the results, MCC had a higher affinity for polyphenolic dye than the regenerated cellulose fibres. Dye uptake on MCC was 50%-80% and 44%-57% on IC. For MCC, the biomordants improved the dye uptake more effectively than the control mordant, alum, whereas for IC the biomordants were less effective than alum.

The pages of this journal attest to the complexity of coloration with natural dyes. Chemists spearheaded the first industrially successful forays into dyeing with synthetic colorants a century and a half ago. At that time, coloration employed time-honoured methods derived from years of trial and error, most of which exploited biological pigments extracted from plant matter. The creators of the early artificial dyes, let alone contemporary dyers who started to move away from processes using natural extracts, stood many decades from possessing a deep knowledge of colorant–substrate interactions at the molecular scale. This relationship is crucial to the design of natural dye-based systems that can challenge established coloration technologies in terms of sustainability, economics and performance. Fortunately, when applied in the right way, modern instrumentation and theoretical frameworks enable researchers to explain the characteristics of plant-derived dyes. Chromatographic analysis coupled to spectroscopy is a powerful tool for deciphering the often complex compositions of natural dye extracts and the impact that colorant preparation procedures make on them. Insight of this kind is all the more important when the components of natural colorants not only exhibit contrasting substantivity and affinity for a fibre, but also interact differently with the mordants purposefully added to the dyebath to increase fastness and alter shade. This Feature article provides a good example of a study which takes such an approach. Its authors utilise chromatography to reveal the behaviour of colourless components in a colorant of plant origin which may indirectly affect coloration by either competing for adsorption to the substrate, oxidising to coloured species, and/or even acting as UV-absorbing photostabilisers. Just as claims that coloration by natural dye systems is more sustainable than that with products of chemical synthesis must be considered on a cradle-to-grave basis, this Feature article illustrates how an extract must be considered as whole when attempting to interpret – and improve upon – its performance.



**Tia Lohtander**, M.Sc. is a doctoral candidate in the Department of Bioproducts and Biosystems at Aalto University. She holds a master's degree in materials science. In her thesis (2018), she explored the use of natural dye from willow bark extract on cellulosic materials. Her current research focuses on creating naturally coloured and functional materials from renewable cellulosic materials and bio-based dyes. She is affiliated to the research group of Functional Cellulose at VTT Technical Research Centre of Finland.

**Suvi Arola** is a senior scientist at VTT Technical Research Centre of Finland in the Functional Cellulose research group. She has a background in biological sciences holding a B.Sc. in biochemistry and M.Sc. in molecular biology. In her dissertation at Aalto University (2015) she explored the nanoscale interaction of biomolecules and nanocellulose materials focusing on surface interactions, rheological properties and nanostructural aspects of materials. Her current work revolves around biochemical modification of lignocellulosic materials and creating novel functional nanomaterials from them utilizing enzymes, proteins and other biological molecules.

**Päivi Laaksonen** works as a principal research scientist at Häme University of Applied Sciences and leads a research team on long term durability of materials. She holds a M.Sc. (2005) and doctoral degree on physical chemistry and since the dissertation at Helsinki University of Technology (2008) she has worked as a senior scientist at VTT Technical Research Centre of Finland and as an assistant professor of materials science at Aalto University, Finland. Laaksonen has studied a wide range of self-assembling nanomaterials including, for instance, metal nanoparticles and biological macromolecules. Currently her interests are applications of bio-based materials in technical and textile materials.

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**ORIGINAL ARTICLE**

Coloration Technology

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**Abstract**

A semi-quantitative study of willow bark dye adsorption on two different cellulose materials using biomordants was carried out. The studied celluloses were microcrystalline cellulose (MCC) AaltoCell and regenerated Ioncell-F (IC) fibres. The dye was a hot water extract of willow bark and the adsorption to cellulose was carried out using carboxylic acid-containing biomordants, namely, oxalic acid, citric acid and tannic acid. Alum was employed as the reference mordant. A semi-quantitative estimation of the dye uptake was conducted using high-performance liquid chromatography equipped with a diode array detector and also by visual inspection, as well as an evaluation of the coloration using CIELab parameters. The mechanism of the dye adsorption on the cellulose surfaces was studied via Fourier Transform–infrared spectroscopy. According to the results, MCC had a higher affinity for polyphenolic dye than the regenerated cellulose fibres. Dye uptake on MCC was 50%-80% and 44%-57% on IC. For MCC, the biomordants improved the dye uptake more effectively than the control mordant, alum, whereas for IC the biomordants were less effective than alum.

## 1 | INTRODUCTION

Wastewater in the dyeing industry constitutes a major source of water pollution globally. The annual production of synthetic dyes is estimated to be 700 000 tons and the related industry grows at an annual rate of 2%.[1,2] In addition to the textiles industry, which consumes *ca*. 70% of the dyestuff produced, the pharmaceutical, food, cosmetics, plastics, ink, paper and photographic industries consume large amounts of synthetic dyes.[3] In textile dyeing, the amount of non-exhausted dye can vary from a few per cent up to 60%, depending on the fibre, dye types and dyeing parameters.[4] Each year, *ca*. 200 000 tons of synthetic dyestuff is lost to effluents in the textiles industry due to incomplete exhaustion and washing operations.[1,5] Effluents containing large amounts of synthetic dyes represent an enormous challenge to wastewater treatment because the chemically stable dyes can readily bypass the conventional wastewater treatment system.[6] In the textiles industry, 60%-70% of dyes are cost-effective azo compounds that possess excellent fastness properties and a wide range of colours.[1,7] However, some azo dyes can have negative effects on health[8,9] and thus the use of aromatic amines releasing azo compounds is restricted.[10]

Biobased dyes offer a potential alternative to synthetic dyes, triggering intensive research during recent years.[11] Natural dyes are reported to be more eco-friendly, less toxic and less allergenic than synthetic dyes.[12] A wide range of dye sources has been explored and their application in conventional textiles,[12] food,[13] cosmetics,[14] dye-sensitised solar cells,[15] as sensitisers in wastewater treatments,[16] in smart packaging[17,18] and functional textiles[19] has been investigated. The shift from synthetic dyes to natural dyes can also benefit other industrial fields, for instance, forest-based industry, as the low-value waste and side streams can be applied in dye production, or the raw materials can be used in new ways which are more profitable.

Willows (*Salix* sp.) are currently utilised as energy crops, but the higher amounts of alkali metals in them, such as potassium and sodium, can reduce their efficiency as they induce slagging, fouling and corrosion in reactors.[20] Thus, novel methods of applying and valorising willow biomass have been of considerable interest. A potential alternative is a biorefinery concept, where the willow crop is fractionised to bark, wood and lignin to produce fibres, sugars and aromatics.[21] In addition, the bark of willow crops contains high amounts of polyphenolic compounds, such as salicin, phenolic glycosides and polymeric tannins, and the content varies between and within species.[22] Willow bark extractives have been previously employed as natural dyes, as tanning agents in leather processing due to their tannin content, and in pharmaceutical products because of their pain- and fever-reducing capabilities.[22-24] In general, different tree barks have been of great interest due to their high availability, and their use for textile dyeing purposes has been investigated.[25] This paper proposes using willow bark extract (WBE) as a natural colorant source for textile dyeing purposes.

The majority of natural dyes have a weaker affinity for adsorption on cellulosic materials, such as cotton and viscose, than on protein-based materials, such as wool and silk, because cellulosic materials acquire a negative charge in water, which causes a repulsive interaction with the dye molecules.[11] Mordants, also known as dye fixatives, are a conventional approach towards improving the dye uptake and fastness properties of dye.[24] Metallic mordants, such as alum and ferrous sulphate, are common in natural dyeing processes owing to the formation of stable coordination complexes and their ability to produce deeper shades than tannins.[26] Major environmental challenges in natural dyeing arise from metallic mordants containing effluents. Due to increased environmental awareness, the use of certain metallic mordants has been restricted.[27] The most common non-metallic mordants are tannins and tannic acid, but metal hyper-accumulating plants and chlorophylls have also been applied.[28,29]

The objective of this study was to investigate the dye uptake of two novel cellulosic materials employing organic biomolecules as mordants and to compare the biomordants to metal mordants commonly used in the process. One of the celluloses was a microcrystalline cellulose (MCC) AaltoCell[30] and the other was a new type of regenerated cellulose fibre, Ioncell-F (IC).[31] The materials were simultaneously mordanted and dyed with WBE. Three different biomordants were compared with the metallic mordant, alum. Dye uptake was quantified using high-performance liquid chromatography (HPLC) with a diode array detector (DAD). Previously, HPLC has not been widely employed in the quantification of dye uptake. However, HPLC coupled to electrospray ionisation mass spectrometry (ESI-MS) has been adopted in the identification of dye compounds in historical textiles.[32] In addition, this method has been employed to quantify the main flavonoids responsible for the yellow colour in weld plants (*Reseda luteola*).[29] The assessment of colour adsorption was also based on the tone that was evaluated, mainly by visual means.

## 2 | EXPERIMENTAL

### 2.1 | Cellulose materials

Never dried MCC, AaltoCell, was prepared from bleached fibres according to a protocol used by Vanhatalo and Dahl.[30] MCC was further diluted to 12 wt% with deionised water. The MCC-water dispersion was treated mechanically three times with dispersioniser equipment (Omega 60 Economic Dispersioniser, Netzsch) to obtain an gel-like material. The processing was conducted at 70 MPa pressure while keeping the cellulose/water mixture under 80°C. The crystallinity index of MCC has been reported as either 50% or 78%, depending on the calculation method used.[30]

Regenerated cellulose fibre bundles were prepared via the Ioncell-F process, which is a Lyocell-type spinning process. The production of IC fibres consists of three main steps: (i) direct dissolution of cellulose with cellulose solvent 1,5-diaza-bicyclo[4.3.0]non-5-enium acetate, (ii) dry-jet wet fibre spinning and (iii) recovery of the cellulose solvent.[31,33] The resulting IC fibres have a crystallinity of 46%-52%.[34]

### 2.2 | Dye materials and chemicals

The dye used in this study was extracted from willow bark (*Salix* sp.). Four-year-old willow hybrids (Karin) were harvested in 2014 in Finland, debarked, and then the bark was dried and stored at 20°C until required. Citric acid (CI, ≥99.5%) oxalic acid (OX, ≥99.0%) and tannic acid (TA, ACS reagent grade) were used as biomordants and were obtained from Merck KGaA. The control metallic mordant, aluminium potassium sulphate dodecahydrate (alum, AL; 98.0%-102.0%), was purchased from Alfa Aesar.

HPLC-grade acetonitrile (≥99.9%) was obtained from VWR International and formic acid was acquired from Merck KGaA. HPLC standards, (+)-Catechin (analytical standard, ≥99.0%) and picein (analytical standard, ≥98.0%), were purchased from Merck KGaA, and triandrin (85%) was acquired from MolPort.

### 2.3 | Dye extraction

The yellow brownish dye was obtained by hot water extraction from willow bark. The extraction was carried out at

80°C for 50 minutes with constant stirring at 250 rpm. The bark-water ratio was kept at 1:20 and the residual solids were removed by crucible filtration twice (VWR qualitative filter paper, particle retention 12-15 μm) and centrifuging (in 50-mL Eppendorf tubes, 4500 *g*, 20 minutes, 21°C). Prior to dyeing, the lyophilised powder extract was stored at −20°C and protected from light radiation.

## 2.4 | Dyeing of cellulosic materials

The AaltoCell (MCC) and Ioncell-F (IC) fibres were dyed with WBE in a hot water bath at 80°C for 60 minutes and the fibres-to-liquid ratio was kept at 1:50. The amount of dye was 20% of the weight of material (owm) and the mordanting was carried out simultaneously with 10% owm concentration. Milli-Q water was used in all reactions. The dyed fibre material and the residual dye solution were separated after dyeing by centrifugation (4500 *g*, 15 minutes, 21°C). The residual dye was collected, lyophilised and stored at −20°C prior to HPLC-DAD-MS analysis. Next, 2 mL of each dyed MCC suspension (2 wt%) was vacuum-filtered into films and both MCC films and IC bundles were dried overnight at 40°C.

## 2.5 | Preparation of HPLC samples

Stock solutions (1000 ppm) of each HPLC standard and dye sample were prepared to 50:50 acetonitrile: Milli-Q water. Stock solutions were diluted for calibration curves to 10-500 ppm and filtered through 0.22 μm Millex-GV filter (Merck Millipore Ltd). A mixture of three standards (1:1:1, 100 ppm) was prepared for investigating the effect of interactions to elution.

## 2.6 | Spectrophotometric measurements

Spectrophotometric measurements of WBE, three standard compounds and four mordants were conducted using a Shimadzu UV-1800 spectrophotometer. The absorption characteristics information of WBE and standard compounds was utilised in developing the HPLC method. Owing to the high absorptivity of all analysed materials in the ultraviolet (UV) spectral range, the measurements were performed separately in both UV and visible ranges. For WBE, four different concentrations between 1.2-3.0 mg/mL were analysed in both measurement ranges. Fourier Transform–infrared photoacoustic (FTIR-PAS) spectra of WBE and dyed and dried cellulose materials were recorded with an FTS 6000 spectrometer (Bio-Rad) and used for obtaining chemical bonding information. The CIELab coordinates and reflectance of dyed samples were recorded using a spectrophotometer (GretagMacbeth Spectrolino) with D65 illumination and 10° observer angle. The colour strength (*K*/*S*) was calculated using the Kubelka–Munk equation:

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

where *R* is the reflectance, *K* is the sorption coefficient and *S* is the scattering coefficient. *K*/*S* was determined at a wavelength of 500 nm from triplicate measurements.

## 2.7 | HPLC-DAD-MS analysis

Liquid chromatography–mass spectrometry (LC-MS) is widely applied in the identification and analysis of food polyphenols and oligomeric proanthocyanidins.[22,35] The quantification of dye adsorption was performed by the HPLC system, 1260 Infinity (Agilent Technologies) equipped with a DAD (G4212; Agilent Technologies), and a quadrupole-time-of-flight mass spectrometer (QTOF MS 6530; Agilent Technologies). The data acquired were processed with MassHunter software version 7.0 (Agilent Technologies). The chromatographic separation method was developed based on a method described by Engström et al.[36] The chromatographic separation was performed with a Phenomenex Kinetex Biphenyl column (100 × 2.1 mm, 2.6 μm, 100 Å). The flow rate of the mobile phase was 0.250 mL/min and the injection volume was 2 μL. The gradient solvent system consisted of 0.1% formic acid in Milli-Q water (A) and in acetonitrile (B). The applied elution profile was as follows: 0-0.5 minutes 95% A:5% B, 0.5-45 minutes from 95% A:5% B to 5% A:95% B (linear gradient), 45-46 minutes 5% A: 95% B and 46-52 minutes 95% A:5% B. The negative ionisation mode was used for ESI-MS analyses. The ESI-QTOF-MS conditions were as follows: gas temperature 350°C, gas flow 11 L/min, nebuliser ($N_2$) 40 psig, capillary voltage 3500 V, fragmentor 150 V, skimmer voltage 65 V and Oct1 RF Vpp 400 V, mass range *m/z* 100-1100.

## 2.8 | Calibration curves

Each standard was analysed in duplicate and the quantitation was performed using DAD. UV spectra were collected at two wavelengths: 260 nm (picein and triandrin) and 280 nm (catechin). Calibration curves were obtained by plotting the concentration against the peak area (the data are listed in Table S1).

The validation of HPLC-MS methods is generally conducted by investigating linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy and reproducibility.[36,37] Picein (100 ppm) standard was used for quality control

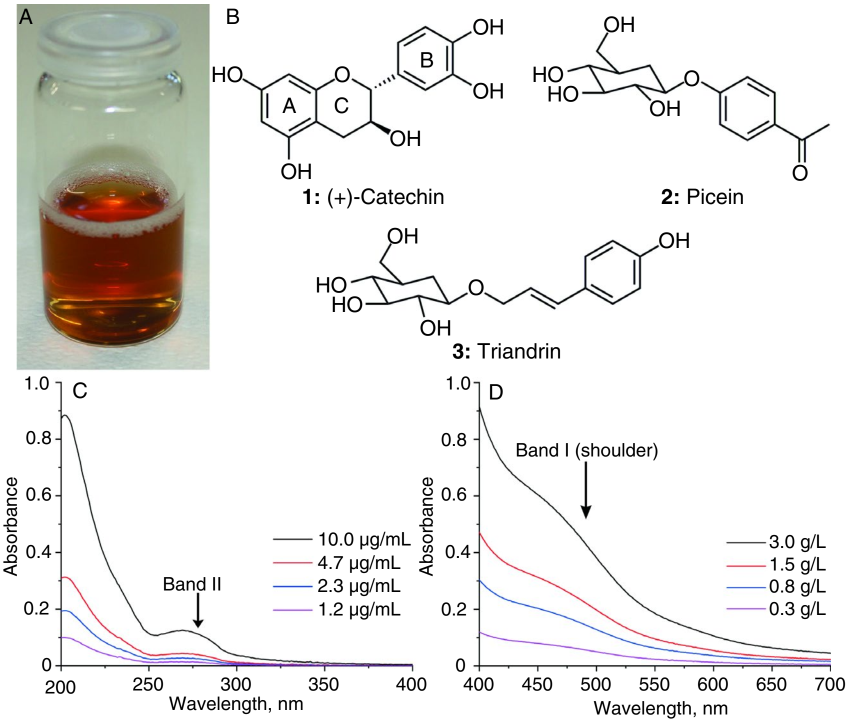


**FIGURE 1** (A) Willow bark extract (WBE) and (B) chemical structures of its three phenolic constituents. Below, ultraviolet (UV)-visible spectra of WBE at different concentrations: (C) in the UV spectral region, and (D) in the visible light spectral region

of the method. Precision was determined by making four replicate measurements carried out within-day, and reproducibility from day-to-day variation, over 5 days. Accuracy was determined by comparing the actual concentration with the concentration according to the calibration curve. LOD and LOQ were determined using Equations 2 and 3, respectively:

$$\mathrm{LOD} = 3\frac{\sigma}{s} \tag{2}$$

$$\mathrm{LOQ} = 10\frac{\sigma}{s} \tag{3}$$

where $s$ is the slope of the calibration curve and $\sigma$ is the standard deviation of the response. The quality control results are given in Table S2.

### 2.9 | Calculation of dye adsorption

The amount of adsorbed dye was estimated by integrating the area under the peaks in the HPLC chromatograms. The area of the peaks in the sample liquids after dyeing the cellulosic materials was compared with the area of the peaks before dyeing, resulting in an estimated dye adsorption percentage. The amount of adsorbed dye (exhaustion%) was calculated by:

$$\text{Absorbed dye (exhaustion\%)} = \frac{(A_0 - A_1)}{A_0} \times 100\% \tag{4}$$

where $A_0$ and $A_1$ are the areas of the chromatogram peaks before and after dyeing, respectively. All of the dye sample measurements were carried out in triplicate and the results are expressed as mean values.

## 3 | RESULTS AND DISCUSSION

### 3.1 | Spectrophotometric characterisation of WBE

Hot water extraction of the willow bark led to a translucent reddish brown solution (Figure 1A). The main components of WBE, (+)-catechin (compound 1), picein (compound 2) and triandrin (compound 3), were identified in an earlier work[38] and are presented in Figure 1B. The WBE had a high absorption in the UV region (Figure 1C), which is characteristic for polyphenolic compounds. All polyphenolic compounds contain at least one aromatic ring (A-ring) and thus have at least one absorption maxima at a spectral range of 240-285 nm (Band II).[39] The second absorption maxima at 300-550 nm (Band I) arises from the conjugation of the B-ring and the C-ring.[40,41] The major ions, chromatograms and individual UV-visible spectra of the main components are presented in Table S1 and Figures S1-S3. Glycosylation, which is present in picein and triandrin, reduced the intensity of the absorption at Band I (Figure 1D).[41]

The FTIR-PAS spectrum of freeze-dried WBE powder (Figure 2) showed bands that are characteristic for phenolic compounds. The broad band at 3371 $\mathrm{cm}^{-1}$ showed the presence of hydroxyl groups and the three bands at 1708, 1512 and 1446 $\mathrm{cm}^{-1}$ arose from carbonyl groups and $CH_2$ and C-C aromatic ring vibrations, respectively. The 1072 $\mathrm{cm}^{-1}$

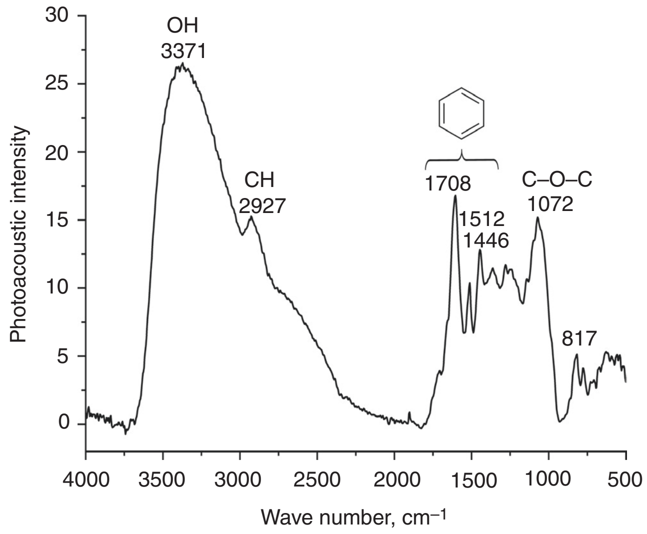


**FIGURE 2** Fourier Transform–infrared photoacoustic (FTIR-PAS) spectrum of willow bark extract (WBE) powder. The spectrum shows bands that are typical for phenolic compounds

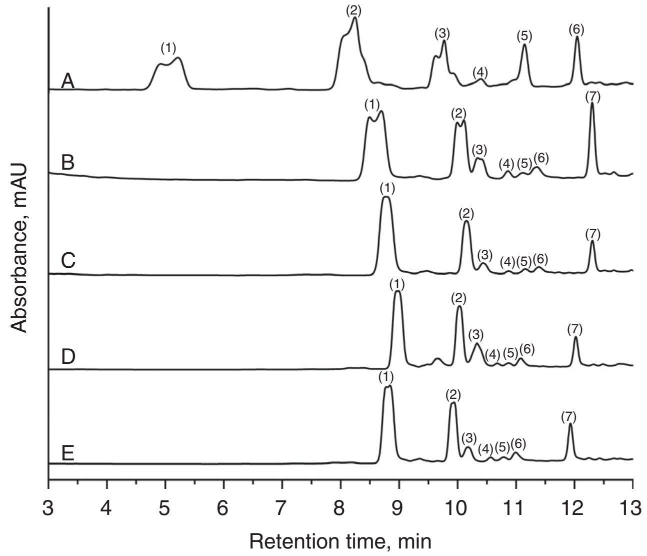


**FIGURE 3** Diode array detector chromatogram spectra of dye and mordant combinations before dyeing: (A) willow bark extract (WBE), (B) WBE + oxalic acid (OX), (C) WBE + citric acid (CI), (D), WBE + tannic acid (TA) and (E) WBE + AL. The composition of each peak was identified based on mass spectrometry (MS) ion peaks. For graphical purposes, each subsequent spectrum is 120 mAU higher than the previous spectrum

absorption band was due to C-O-C and the band at 817 $cm^{-1}$ showed substitutions of aromatic rings.[42]

## 3.2 | Composition of the WBE solutions

The composition of the WBE solutions was studied by separating the individual components using LC and analysing the mass of the individual peaks separately. The HPLC-DAD spectra of WBE (Figure 3, chromatogram A) showed six absorbance peaks and the composition of each peak was identified based on MS information. The major peak (1) contained picein. Peak (2) contained a mixture of all the main components, and peak (3) consisted of a mixture of triandrin and catechin. Ion *m/z* 179 was present in peaks (2) and (3) and the mass matched with monosugar. The peaks (4) and (5) contained both monomeric and dimeric ions of catechin, but from peak (6), only dimeric catechin was identified. The peaks (5) and (6) also contained an unidentified fragment ion *m/z* 135.

Before the dyeing experiments, the solutions containing mordants were also analysed to identify possible interactions between the mordants and the dye molecules. The chromatograms are shown in Figure 3. The composition with the mordants had similar features to the WBE without any mordant, except for those solutions containing oxalic acid, which showed a distinctly different profile to the others, as the three first peaks at retention time (RT) 8.5, 10 and 10.2 minutes displayed notable peak-splitting. In the presence of tannic acid, the absorbance peak at 10.5 minutes had a greater intensity than in the other three spectra. This was probably due to the strong absorption of tannic acid close to the detection wavelength of 280 nm, which can affect the overall intensity of the chromatogram. In ESI-MS, the absorbance peak at 10.5 minutes differed from other spectra with ion *m/z* 321. According to a previous study, ion *m/z* 321 is typical for tannic acid, which consists of esterified central glucose molecules and gallic acid units, and can be attributed to a dimer of gallic acid.[43] The UV-visible spectra of the mordants and the composition of each peak in the chromatograms are given in the Figures S4 and S5, and Table S3.

In the LC the compounds often co-eluted, which prevented the quantification of individual compounds. The observed co-elution arose from the instability and structural similarity of the phenolic compounds present in WBE. Monomeric catechins can also form dimers and polymeric proanthocyanidins (condensed tannins) via three different reaction sites (A-ring C-8 and C-6, B-ring C-6).[44] According to the MS, dimers of catechin (*m/z* 577 [2M-3H]) and picein (*m/z* 595 [2M-H]) were present in the extract, showing that dimerisation can occur under the applied conditions. Catechin, as well as other polyphenols, is known to be susceptible to oxidation, which can be induced enzymatically or non-enzymatically (ie, due to oxygen, temperature, pH and light).[44,45] The structural changes can have an effect on chromatographic separation and thus cause uncertainties in the quantitative analysis of WBE. As well as the reactivity of polyphenols, problems arising from the system, such as the matrix effects and ionisation competition between co-eluted molecules,[35] can present challenges to the quantification with ESI sources.

## 3.3 | Dyeing of cellulosic materials

The MCC and IC samples after dyeing with different dye and mordant combinations are presented in Figure 4, including

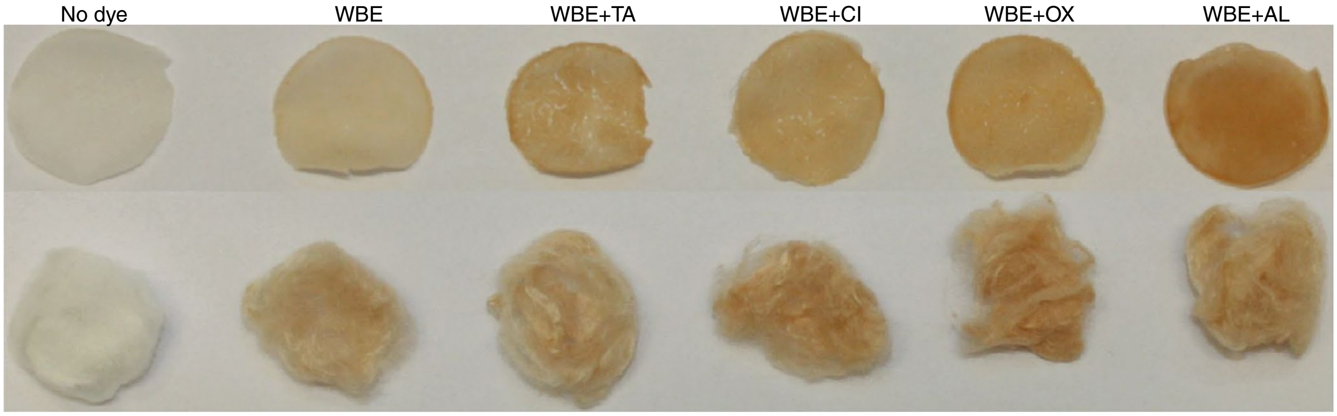


**FIGURE 4** Microcrystalline cellulose (MCC) (top row) and Ioncell-F (IC) (bottom row) samples after dyeing under different dyeing conditions with willow bark extract (WBE), WBE + tannic acid (TA), WBE + citric acid (CI), WBE + oxalic acid (OX) and WBE + alum (AL)

**TABLE 1** *K*/*S* and CIELab coordinates of microcrystalline cellulose (MCC) and Ioncell-F (IC) samples on their own and after dying with willow bark extract (WBE), WBE + alum (AL), WBE + citric acid (CI), WBE + oxalic acid (OX), WBE + tannic acid (TA)

| Sample | *K*/*S* | *L** | *a** | *b** |
|---|---|---|---|---|
| MCC | 0.1 | 87.5 | −1.0 | 8.2 |
| MCC + WBE | 0.3 | 81.6 | 3.3 | 20.6 |
| MCC + WBE + AL | 2.1 | 59.9 | 16.3 | 37.0 |
| MCC + WBE + CI | 0.7 | 73.9 | 9.5 | 31.1 |
| MCC + WBE + OX | 0.8 | 72.7 | 11.0 | 32.5 |
| MCC + WBE + TA | 0.2 | 81.0 | 4.6 | 18.2 |
| IC | 0.1 | 89.0 | −0.1 | 7.6 |
| IC + WBE | 0.6 | 74.9 | 8.2 | 25.3 |
| IC + WBE + AL | 1.1 | 67.6 | 10.2 | 28.1 |
| IC + WBE + CI | 1.1 | 68.5 | 11.4 | 29.1 |
| IC + WBE + OX | 1.6 | 63.3 | 13.8 | 31.9 |
| IC + WBE + TA | 0.8 | 71.6 | 9.2 | 26.2 |

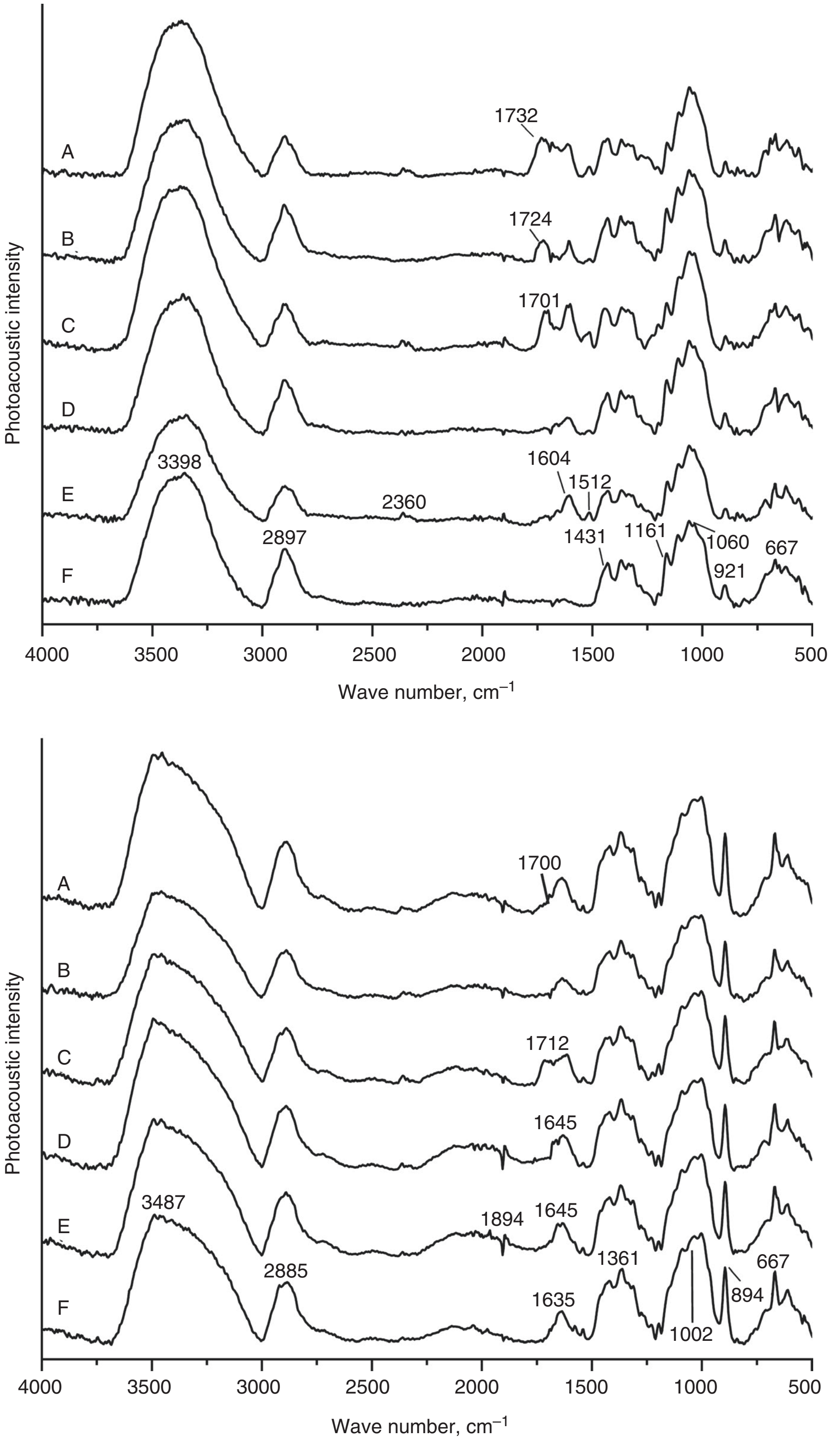
the undyed control samples. The *K*/*S* values and CIELab parameters are listed in Table 1. Visual inspection of the results and *K*/*S* values showed that for MCC the resulting colour intensity was highest with alum mordanting, in comparison with those samples dyed with other mordants. For IC, the most intensive colour was obtained with citric acid, oxalic acid and alum mordanted samples, and the differences between them were small. Dyeing without mordant yielded the lightest results for both materials. Evaluation of the visual appearance, however, was limited to only the visible spectrum of colours, and therefore further investigation of the dye uptake was carried out, as described later.

The dyeability of materials is known to depend on the number of available adsorption sites and the ratio of crystalline and amorphous regions on the dyed surface. More loosely arranged amorphous regions are more readily dyed than the crystalline regions.[46] Depending on the crystallinity index calculation method adopted, the degree of crystallinity of MCC (50% or 78%) is either higher than or equal to IC (46%-52%).[30,34] However, in this study, the shades obtained were darker with MCC than IC, which may have been due to the dyeing conditions and the differing appearance of the samples. The IC fibres were rather shiny, whereas the MCC samples had a smaller reflecting surface. As the dyeing of MCC took place in suspension, the distribution of MCC fibres and the dye was more uniform than in IC reactions. Also, the openness of the fibre structure affects the sorption of water and dye molecules. MCC has high porosity because the cellulose material remains undried during the production process and no hornification takes place.[47] Lyocell-type fibres, such as IC fibres, are continuous filaments with a homogeneous fibrillary fibre body and only have a few small voids.[48]

FTIR spectra of undyed and dyed MCC and IC are presented in Figure 5. For both materials, the spectra showed characteristic bands of cellulose fibres. The wide band at 3398 cm$^{-1}$ could be attributed to vibrations of hydrogen-bonded OH groups, 2897 cm$^{-1}$ was due to C-H stretching, and 1431-1419 cm$^{-1}$ indicated C-H asymmetric deformation. The bands between 1161-921 cm$^{-1}$ arose from vibrations of polysaccharides, such as glucose ring stretching and C-O stretching.[49] The spectra also revealed the differences in the cellulose crystal lattice structures of both MCC and IC. MCC had a band at 1431 cm$^{-1}$, which indicated that fibres contained a significant amount of cellulose I, whereas in IC the band shifted to 1419 cm$^{-1}$, which was attributed to cellulose II. In IC, the band at 894 cm$^{-1}$ was characteristic of regenerated cellulose fibres.[50]

After dyeing (Figure 5A-E) the spectra had additional bands, indicating the presence of the dye and the mordants. All of the dyed MCC samples had bands at 1604 and 1512 cm$^{-1}$, which were mainly assigned to the vibrations of the aromatic rings of WBE. Different mordanted samples also had a specific band or bands present in their spectra.

**FIGURE 5** Fourier Transform–infrared (FTIR) spectra of (top) microcrystalline cellulose (MCC) samples and (bottom) Ioncell-F (IC) samples: (A) willow bark extract (WBE) + oxalic acid (OX), (B) WBE + citric acid (CI), (C) WBE + tannic acid (TA), (D) WBE + alum (AL), (E) WBE and (F) no dye. For graphical purposes, each subsequent spectrum is 10 units higher than the previous spectrum



In the spectra of MCC mordanted with citric acid and oxalic acid, bands at 1724 and 1732 cm$^{-1}$ were obtained, respectively. It has been reported that a band at 1737 cm$^{-1}$ may be assigned to the formation of ester carbonyl groups after treating cellulose with concentrated oxalic acid. In the previous study, however, the authors were able to detect a moderate level of esterification after treatment under harsh conditions, casting doubt on our possible observation of esterification in the current study.[51] The bands that were obtained for MCC are close to the reported wavenumber and therefore may be a result of the reaction between mordants and the cellulose surface.[51,52] IC also had a band at

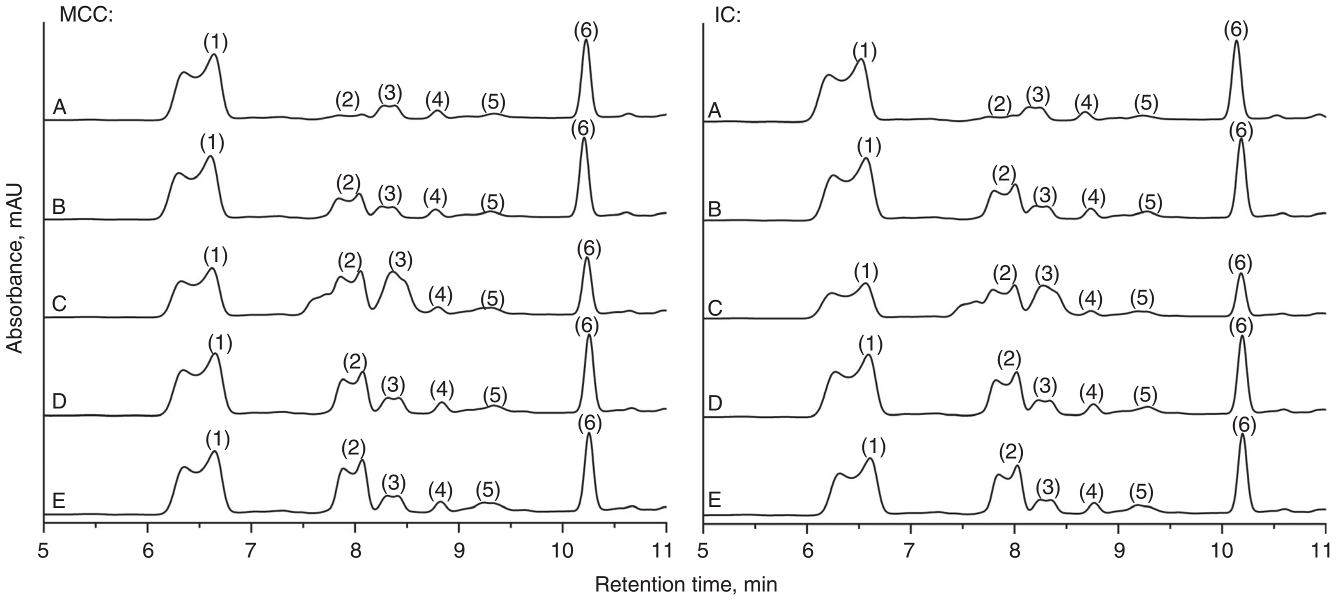


**FIGURE 6** Diode array detector chromatograms of willow bark extract (WBE) and mordant combinations after dyeing microcrystalline cellulose (MCC) and Ioncell-F (IC): (A) WBE + oxalic acid (OX), (B) WBE + citric acid (CI), (C) WBE + tannic acid (TA), (D) WBE + alum (AL) and (E) WBE. For graphical purposes, the WBE-before sample was increased by 120 mAU in each subsequent spectrum

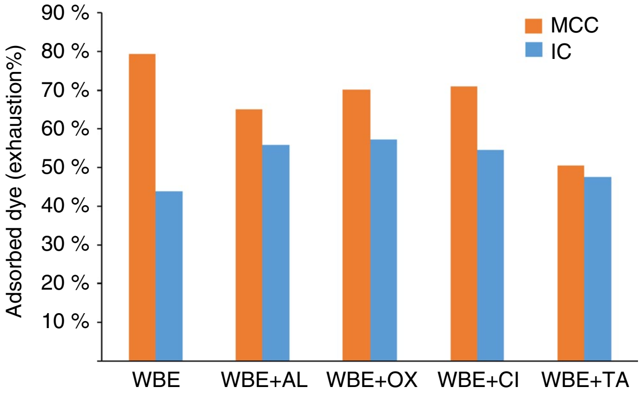


**FIGURE 7** The percentage of adsorbed dye in each experiment. The estimation was performed by comparing sample liquids with the original willow bark extract (WBE). The mordants were oxalic acid (OX), citric acid (CI), tannic acid (TA) and alum (AL)

1635 $\text{cm}^{-1}$, which overlapped with the band arising from the vibrations of aromatic rings of WBE. Thus, only small changes were detected in the spectra of dyed IC samples A-E compared with the original IC in spectrum F. This implies that the dyeing of IC was mainly due to physical adsorption, and not chemical bonding of the dye or the mordant.

## 3.4 | Dye adsorption efficiency

In addition to the visual inspection, dye adsorption was also investigated by analysing solutions before and after dyeing using HPLC-DAD-MS. Because it was not possible to fully separate the individual WBE components due to the co-elution, the sum of the areas under the peaks in the chromatogram were used as an estimate for the total amount of dye. The DAD chromatograms at 280 nm for different dye and mordant combinations after dyeing both MCC and IC are shown in Figure 6. The compositions of the dye liquids after dyeing MCC and IC are presented in Table S4, and the total peak areas for each sample are presented in Tables S5-S7. For simplicity, the obtained peak areas were always compared with the as-prepared WBE solution before dyeing. The results are summarised graphically in Figure 7 showing the percentage of adsorbed dye (exhaustion%) in each experiment.

The results obtained from comparing the total peak areas showed that dye adsorption was generally higher for MCC than for IC samples. This result is in agreement with the visual observations made, where the series of dyed MCC samples had a more intense colour (Figure 4) than that of IC samples. For dyeing MCC, the best mordants were citric and oxalic acid, whereas for IC fibres the best results were obtained with alum, oxalic acid and citric acid, each of which yielded an approximately similar dye uptake. Tannic acid gave the weakest dyeing result with both cellulosic materials, which was surprising as tannins have been the most widely applied mordant historically. Tannins are known to have good affinity towards fibres and they enhance the affinity between fibres and natural colorants due to an increased number of hydroxyl and carboxyl groups in the fibre.[46] As mentioned earlier, the strong UV absorption of tannic acid may affect the accuracy of the DAD analysis, and this may therefore be the reason for such an unexpected result.

When comparing the visual observations, the results obtained from the DAD semi-quantification were different to those obtained within the dyeing series. According to the visual observations made, the best dyeing results for MCC were obtained with alum, and the lightest with WBE without mordanting. However, the dye adsorption estimation based on changes in the total peak areas showed that the largest changes in the quantities of the chemical compounds were in WBE without mordant and in WBE mordanted with oxalic acid. The difference in the results was probably a consequence of different spectral regions being investigated. Whereas the colour observed with the naked eye is based on absorption within the visible light spectral range, the HPLC-DAD semi-quantification was performed within the UV light range. Even although they were not visible to the naked eye, the UV-absorbing compounds involved in the dyeing process may be an important factor in the dyeing result, for instance, by enhancing the colour stability attributed to UV-protection.[53]

Some differences in the adsorption of catechin, picein and triandrin were observed in the quantitation. The DAD chromatograms of dye liquids after dyeing MCC and IC revealed that the absorbance of both oxalic and citric acid mordanted samples showed a lower intensity in peaks (2)-(5) than observed with the other combinations. In particular, peak (2) in the sample WBE + OX was barely detectable compared with the other dye combinations. According to ESI-MS, the absorbance of peak (2) in the sample liquids collected after dyeing was attributable to triandrin. However, in the sample WBE + OX, ions of triandrin were not detected. Therefore, it can be concluded that carboxylic acid-based mordants were able to encourage a better uptake of triandrin on MCC and IC than the other mordants could. From all three polyphenolic compounds present in WBE, triandrin has the most intense colour (Figure S3, panel B). This finding is in agreement with visual observations made as oxalic and citric acid mordanted samples yielded intense colours. Adsorption of picein [peak (1)] was highest in the case of MCC without mordant. In IC, the differences in the peak areas with different mordants were smaller than in MCC; in MCC, citric acid yielded the greatest change. Carboxylic acid-based mordants also efficiently adsorbed catechin, which was detected in peaks (3)-(5). Catechin is a colourless compound and therefore it does not have a direct effect on the colour of MCC and IC. However, as catechins are known to be susceptible to oxidation and polymerisation, yielding to a browning of colour,[44] therefore the amount of adsorbed catechin can have an indirect effect upon the intensity of colour.

## 4 | CONCLUSIONS

The dyeing capability of WBE on MCC AaltoCell and regenerated IC fibres was compared using both a visual inspection and a semi-quantitative HPLC-DAD-MS method. The chemical changes on the cellulose surfaces in the dyeing process were analysed by FTIR. The efficiency of three carboxylic acid-containing biomordants as dye fixatives was evaluated and compared with that of a metal mordant, alum. In general, the dye uptake on MCC was higher than on the regenerated IC. With the biomordants tannic acid, citric acid and oxalic acid, FTIR analysis indicated chemical bonding of the mordant on the MCC surface. For IC there were no signs of chemical modification. The efficiency of the mordants in terms of dye uptake varied only slightly, and the biomordants showed comparable results with the commonly applied alum. The results demonstrated that oxalic and citric acid are suitable alternatives to alum when cellulosic fibres are dyed with polyphenolic WBE.

The results of the visual observations and the HPLC-DAD semi-quantification were partially inconsistent due to the different spectral regions that the analysis was based upon. Dyes extracted from natural sources are often mixtures of multiple components that may have differing spectral properties, and which are not always revealed in the visual inspection. Some polyphenolic compounds may only absorb UV light, and while adsorbing on to the fibres they are blocking the adsorption sites to coloured components. The comparative analysis carried out in this study highlights the importance of quantifying all of the compounds of nature-derived dyes in order to understand the results of dyeing experiments. Our findings provide encouragement to find alternatives to synthetic dyes from nature, and for further developing natural dyeing processes so as to be more environmentally friendly. The next step for future research would be to develop the dyeing process to a larger scale and to minimise water usage.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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