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Natural dyes in madder (Rubia spp.) and their extraction and analysis in historical textiles

Richard S. Blackburn
School of Design, University of Leeds, Leeds, LS2 9JT

Abstract

Textiles coloration using extracts from the roots of various madder species (Rubia spp.) has been performed for centuries. To date, 68 anthraquinone colorants have been detected in Rubia spp. used to dye textiles. Many of these dyes are sensitive to hydrolysis and degradation from enzymes, extraction chemicals and processing temperatures, and are often overlooked as colorants in historical textiles. Conclusions in literature of the past 30 years concerning colorants present in planta and, particularly, in madder-dyed artefacts are being challenged as new analysis methods are developed. The recent advent of 'soft' extraction techniques has demonstrated that anthraquinone glycosides and other sensitive molecules, such as carboxylated compounds, need to be preserved; this valuable chemical information embedded in the dye structure may be lost if extraction and analysis is too harsh. Some compounds thought to be present in madder and madder-dyed artefacts are in fact degradation products resultant from the extraction process, and degradation pathways have been developed to better understand the reactivity and stability of these compounds. Detailed analysis of dyes in textile artefacts can reveal important cultural and heritage information concerning historical textiles relative to the specific dye species, the area of the world where this may have grown, how and where it was dyed, and, perhaps, where it was traded. Understanding the precise molecular structure of these dyes and their chemical reactivity is important to provide knowledge of their interactions with physical substrates, such as textile fibres, which could be used to develop superior techniques for analysis of artefacts.

Keywords: anthraquinone; soft extraction; chromatography; history; artefacts.

1. Introduction

Throughout history textile materials have been dyed with colorants extracted from various plant and animal species. Many examples of dyeings remain today in museum and conservation collections, particularly in the UK. These textile artefacts are more than just a snapshot of what the colours of our history might have looked like, they can also reveal important information about our cultural past and heritage. Often, natural colorants are complex mixtures of many different molecules and the nature of the colorants present, chemical structural differences, and the ratio of the abundance of these molecules can provide significant information about which plant species was used to dye the fibres or the technique used for the dyeing process. In the context of historical textiles, this information is of paramount importance for conservation and restoration purposes, as well as the generation of information on the ethnographic origins of the artefacts. The greater the level of understanding that can be gained about the colorants (or their degradation products) present in textile artefacts, the more information is obtained from biological and chemical perspectives, which has significant ethnographic implications. To truly understand our heritage, questions beyond the basic colour of an artefact must be answered. Which particular plant or animal species was used as the source of the dye? Where in the world might this plant or animal species have grown or been present? What geographic routes might have operated for trade of these dye-containing materials and where were they used for dyeing? Where in the world were such dyed articles traded? When and how did all these various dyeing activities take place? These questions can only be addressed through detailed chemical and biochemical analysis of textile artefacts using the vast array of analytical tools available today often in combination with techniques to effectively extract the dyes from the substrate to which they have been applied. Understanding the nature of the dyes contained in artefacts, and the associated colour changes and dye degradation processes that may occur in the dyeings, can assist the conservator with interventive treatments and preventive conservation strategies to counteract such fading and ageing.

2. Colorants present in planta in different Rubia species

Colorants extracted from the roots of various plant species of the genus Rubia have been used as dyestuff for centuries, and have been generally grouped together under the broad name of ‘madder’, although this actually represents several identified species from origins all over the world (Table 1). Dioscorides wrote about madder’s usefulness in dyeing red and the dyestuff was well known to the dyers of Egypt, Greek and Roman eras [1]; however, only in the 16th Century did dyeing recipes start to be readily available in literature [2]. Dyers understood that the various madder types could provide different shades, but a knowledge of the reason for this was not available until the 19th century.

Table 1. Plants from various Rubia species used historically for dyeing textiles. Taxonomy verified according to Kew Gardens Medicinal Plant Names Services database [3]. The Colour Index name provided is the mixture of colorant compounds extracted from the roots of the plant.

Plant common name	Plant species	Scientific synonyms	Colour Index Name
Dyer's madder	Rubia tinctorum L.	R. acaliculata; R. iberica; R. sativa; R. sylvestris	C. I. Natural Red 8
Wild madder	Rubia peregrina L.		C. I. Natural Red 8
Indian madder	Rubia cordifolia L.	R. oncotricha; R. manjith; R. munjista; R. sylvatica	C. I. Natural Red 16
Naga madder	Rubia sikkimensis Kurz		None
Xiao hong can (no English name)	Rubia yunnanensis Diels	R. ustulata	None
Japanese madder	Rubia akane Nakai	R. argyi	None

In 1826, Robiquet & Colin reported that R. tinctorum root contained two colorants, alizarin (**2**) and the more rapidly fading purpurin (**11**) [4,5]. The majority of 19th and 20th century literature that followed similarly concluded that the major colorant present in extracts from R. tinctorum was alizarin, and the primary colorant present in R. cordifolia was purpurin. However, plant biosynthesis typically results in both compounds with sugar moieties attached (glycosides) and the same parent structure without sugar moieties (aglycons); glycoside derivatives have increased water solubility over their aglycon counterparts. The 2-O-linked disaccharide of alizarin, ruberythric acid, was actually first isolated from an unspecified species of madder by Rochleder in 1851 [6], but it is only relatively recently that significant evidence has confirmed the primary anthraquinone components in R. tinctorum roots are the glycosides ruberythric acid (**1**) and lucidin primeveroside (**3**) [7-11]. Whilst the aglycon alizarin does occur in the plant, it is in much lower concentrations than its glycoside; total glycosides present in R. tinctorum roots are in at least a five-fold concentration over total aglycons, with individual glycoside concentrations significantly higher than their aglycon counterparts [9,11]. Despite alizarin being particularly synonymous with "madder" there is strong evidence that it, and ruberythric acid, only occur in significant concentrations in R. tinctorum [12].

Singh et al. [13] report 68 anthraquinonoid compounds to be extractable from madder roots of the various species, although many of these compounds are artefacts of the inherent reactivity of some of the colorants during analytical extraction methods, and are suspected as not being not present in planta; for example, anthraquinones that contain a 2-methoxymethyl- or a 2-ethoxyethyl group are formed during extraction with hot methanol or ethanol, respectively [7,14].

Table 2. Compounds detected in the six main Rubia species studied in literature. ++ indicates major compound in planta, + significant amount, tr. very low or trace amount.

Compound	Mass (Da)	R. tinctorum	R. cordifolia	R. peregrina	R. sikkimensis	R. yunnanensis	R. akane
ruberythric acid (alizarin primeveroside) (**1**)	534	++ [8-12,15-18]	tr. [18]				
alizarin (**2**)	240	+ [7-12,15-19]	tr. [18,19]	tr. [18]			
lucidin primeveroside (**3**)	564	++ [7-11,16,18]	tr. [18]	++ [12,20,21]	+ [12]	+ [22]	
lucidin (**4**)	270	tr. [8,9,16,18,23]		+[21]			
nordamnacanthal (**5**)	268	+/tr. [8,12,16,18,24]		+ [12]			
munjistin (**6**)	284	+ [8,12,18,19]	++ [12,15,19,25]	+ [12,19]	++ [12,26]	+ [12,22]	++ [12]
xanthopurpurin (**7**)	240	+/tr. [8,12,16,17,18,27]	+ [12,18,25,28]	+ [12]	+ [12]	+ [22,29]	tr. [12]
galiosin (pseudopurpurin primeveroside) (**8**)	594	+ [8,12,15]		++ [12,15,21]			tr. [12]
pseudopurpurin glucoside (**9**)	462	+ [8,12]	+ [12]				+ [12]
pseudopurpurin (**10**)	300	+ [7,8,12,15,17-19,27]	++ [15,30]	++ [12,15,19]	++ [12]	+ [12]	++ [12]
purpurin (**11**)	256	+/tr. [8,11,12,15,16,18,19]	+ [12,19]	++ [12,19]	+ [12]		tr. [12]
rubiadin primeveroside (**12**)	548	+ [9,15]		+/tr. [12,20,21]			
rubiadin (**13**)	254	+/tr. [12,16,18,19]	tr. [18,19,25,28]	+ [12,20]		+ [22,29]	
1-hydroxy-AQ (**14**)	224	+ [17]	+ [31]				
1-hydroxy-2-methyl-AQ (**15**)	238	+ [18,27,32]	+ [18,24,25,28]	+ [20]		+ [22,29]	+ [18]
1-hydroxy-2-hydroxymethyl-AQ (**16**)	254		+ [25,33]				
1-hydroxy-3-carboxy-AQ (**17**)	268		+ [18,34]				
1,4-dihydroxy-2-hydroxymethyl-AQ (**18**)	270		+ [34]			+ [22]	
1,4-dihydroxy-2-methyl-AQ (**19**)	254		+ [28]				
anthragallol (**20**)	256	+ [27]					
2-hydroxy-AQ (**21**)	224	+ [27]					
2-(hydroxy methyl)-AQ (**22**)	238	+ [27]				+ [22,29]	
2-(hydroxy methyl)-3-hydroxy AQ (**23**)	254					+ [29]	
6-hydroxyrubiadin-3-O-neohesperidoside diAc (**24**)	662		+ [18,25]				+ [12]
6-hydroxyrubiadin-3-O-neohesperidoside monoAc (**25**)	620		+ [12,18,25,35]		+ [12]	++ [12,22,29]	++ [12]
6-hydroxyrubiadin-3-O-neohesperidoside (**26**)	578		+ [12,18,25]		+ [12]	++ [12,22,29]	++ [12]
6-hydroxyrubiadin-3-O-glucoside monoAc (**27**)	474		+ [18]			+ [29]	
6-hydroxyrubiadin-3-O-glucoside (**28**)	432		+ [18,25]				
6-hydroxyrubiadin (**29**)	270		+ [18,25,33]			+ [12,22,29]	
rubianine (**30**)	402	+ [36,37]					
munjistin-3-O-glucoside (**31**)	446		+ [12]				++ [12]

O-primeveroside, O-(6-O-β-D-xylopyranosyl-D-glucopyranosyl); O-neohesperidoside, O-(2-O-α-L-rhamnopyranosyl-D-glucopyranosyl); AQ, anthraquinone; diAc, diacetate; monoAc, monoacetate.

Table 2 summarises the compounds detected in various studies on the six main Rubia species in previous studies (excluding products of reaction with the extraction solvent). Many of these compounds may not be present, or present in low concentrations, in planta; for example, R. peregrina

only contains very low levels [15,38] and sometimes no [21] alizarin or ruberythric acid. Other compounds that have been identified may actually be a result of harvesting, drying, or processing of the madder root before it is used for dyeing, and the levels of the anthraquinonoid compounds present may vary with age [39].

Anthraquinone O-glycosides can be hydrolysed to their aglycon counterparts (Figure 1); this may occur in the roots of the plant catalysed by endogenous enzymes, but could also be during chemical processing, especially under acidic conditions. Schunck demonstrated that ruberythric acid (**1**) could be hydrolysed to alizarin (**2**) in planta, catalysed by one of the first enzymes ever studied, which he isolated and called "erythrozyme" [40]; this is most likely via the glucoside, although isolation of the glucoside has not been reported. Hill & Richter [15] demonstrated that boiling madder roots in water destroyed (denatured) the hydrolytic enzymes and the glycosides could be readily extracted. They also found that the stability of different anthraquinone glycosides varies and demonstrated that galiosin (**8**) is very unstable and is readily hydrolysed to pseudopurpurin (**10**), which is as a result of glycoside substitution at the α-hydroxy in the 1-position; ruberythric acid (**1**) and rubiadin primeveroside (**12**) have glycoside substitution at the β-hydroxy in the 2-position, making them significantly more stable to hydrolysis; the presence of a carboxylic acid moiety within galiosin at the 2-position may also catalyse hydrolysis. Mouri & Laursen [12] recently confirmed that, unless R. tinctorum roots were "warmed in water" for prolonged periods (hence, providing enzymatic incubation conditions), significant concentrations of anthraquinone glycosides were present in the dyebath and on dyed wool fibre. They demonstrated that steaming madder roots or boiling them in water for 30 seconds was sufficient to deactivate the hydrolytic enzymes. An initial extraction process by boiling the madder root was typically performed in the Japanese Kusaki-zome dyeing method [41]; typical European madder dyeing processes historically involved heating the dyebath to 75-80 °C [42], which would most probably also denature the endogenous enzymes, ensuring any glycosides present in the root were also present in the dyebath. Ford et al. [16] also demonstrated in analysing three varieties of R. tinctorum that drying the roots with heating also ensures that glycosides are present in the root, in much higher concentrations than aglycons, are present in the dyebath, and are the primary dyeing species observed on the fibre, not alizarin; this was also observed in aged samples.

Interestingly, despite high concentrations of lucidin primeveroside (**3**) in R. tinctorum roots [9,11,12], the aglycon lucidin (**4**) is rarely detected (and then only in low and trace concentrations in planta and in textile artefacts [8,9,12,23]) even when acidic conditions are used that would promote hydrolysis (**3**→**4**); it is suspected that the reactive nature of lucidin means that it is readily converted to other compounds. As Figure 1 shows, lucidin (**4**) can be oxidised to nordamnacanthal (**5**), and studies have suggested this is catalysed by endogenous oxidase enzymes in the plant [7,43,44].

Figure 1

Munjistin (**6**) is often cited as the most abundant colorant present in the roots of both R. cordifolia and R. yunnanensis [12,22,25], and is also observed in several other Rubia species [8,12]. However, a glycoside (munjistin-3-O-glucoside; **31**) has only ever been detected in R. cordifolia and R. akane [12], suggesting that munjistin is formed in planta (especially in other species) through a different mechanism. It is possible that nordamnacanthal (**5**) can be further oxidised to form munjistin; in contrast, Hill & Richter [15] proposed that munjistin (**6**) was formed by natural reduction of pseudopurpurin (**10**), although this seems less likely. Xanthopurpurin (**7**) is formed through decarboxylation of munjistin (**6**) [45]; xanthopurpurin may also be formed directly from lucidin (**4**) through an acid (or base)-catalysed loss of formaldehyde through a retro-aldol type process (Figure 1).

After munjistin, purpurin (**11**) is often cited as the most abundant colorant present in R. sikkimensis, R. cordifolia and R. akane [12]; it is also identified in R. tinctorum [8,11,12,15,16,18,19]. However, it is suggested that purpurin is formed through decarboxylation of pseudopurpurin (**10**) during drying of the roots [45]; pseudopurpurin is found as a significant component in the roots of all Rubia species presented herein [7,8,12,15,17,27,30]. It is also likely that the pseudopurpurin glycosides galiosin (**8**) and pseudopurpurin glucoside (**9**) occur in planta – their presence has been detected in low concentrations in R. tinctorum [8,12,15], R. peregrina [12,15,21], and R. akane [12], and are most probably the origin of pseudopurpurin (**10**) as a result of hydrolysis (**8**→**9**→**10**) [7-9,12]. However, there is no literature evidence of the presence of pseudopurpurin glycosides in R. cordifolia.

Rubiadin primeveroside (**12**) occurs in very low concentrations and has only ever been detected in the roots of R. tinctorum roots (<2%) [9,15] and R. peregrina [20,21], and is most likely the origin of trace amounts of rubiadin (**13**) detected in those two species [12,16,20] as a result of hydrolysis (**12**→**13**). Rubiadin has also been observed in low concentrations in R. cordifolia [25,28] and R. yunnanensis [22,29], and although rubiadin primeveroside has not been detected in these species, it is most likely the source. Several other simple aglycon anthraquinone derivatives (**14-23**) have been observed in low concentrations in Rubia species.

Structures 14-23

6-hydroxyrubiadin (**29**) and its glycosides are not found in R. tinctorum and R. peregrina, but have been detected in the Asian madder species R. cordifolia [18,25,33,35], R. sikkimensis [12], R.

yunnanensis [12,22,29], and R. akane [12]. It is additionally interesting that 6-hydroxyrubiadin glycosides are the only glycosides present in Rubia spp. that are acetylated [12,18,22,25,35]; it is not known whether the various diacetates, monoacetates and non-acetylated forms are all biosynthetically produced in planta or whether they are artefacts of deacetylation (**24**→**25**→**26** or **27**→**28**); acetyl groups may be removed chemically, but acetylesterases that would catalyse the enzymatic conversion of an acetate to an alcohol are known, although they have not been identified in Rubia spp. Enzymatic or chemical hydrolysis of 6-hydroxyrubiadin neohesperidosides to their respective glucosides (**25**→**27** or **26**→**28**), and ultimately aglycon (**27**→**29** or **28**→**29**), is also possible.

Despite all other anthraquinone glycosides found in various madder species being the O-glycoside, Schunck claimed to have isolated the C-glycosyl anthraquinone "rubianine" from the roots of R. tinctorum in 1893 [36], the structure of which was confirmed by ^{1}H and ^{13}C NMR spectroscopy almost 100 years later [37]; rubianine (**30**) is essentially the 2-C-glucoside of xanthopurpurin.

Structures 24-31

3. Extraction and identification of colorants in textile artefacts dyed with different Rubia species

In the context of historical textiles, detailed information on the complex mixture of natural colorants present in the artefact is of paramount importance for conservation and restoration purposes, as well as the generation of information on the ethnographic origins of the artefacts. When extracting and analysing colorants from textile artefacts as much information should be preserved as possible in order to gain better insight on how they were dyed and the plant species from which the dye originated, hence, it is important to limit the damage to the colorant molecule in the analysis process. Chromatographic techniques have become important tools in analysing samples of historical interest due to the complex mixtures of colorants involved when dyeing with natural dyes [46,47]. However, extraction of artefacts is not straightforward as the dyes are strongly bound to the substrate via a mordant metal.

3.1. Metal complexes formed in mordant dyeing with madder

Throughout history, when dyeing with madder a mordant was required to expedite dye adsorption and fixation onto the fibre. Mordants are typically inorganic metal salts which dissociate in solution to become a metal ion that forms a ligand complex between dye and fibre. Before application of the dye to the fibre, the fibre is pre-mordanted, traditionally with hydrated potassium aluminium sulfate ($KAl(SO_4)_2 \cdot 12H_2O$), which is referred to in literature as alum or potash alum [48]. These metal ions

(Al^{3+} in the case of alum) act as Lewis acids in solution, the positive charge of the central metal ion draws electron density from an O–H bond on a water molecule coordinated to it and the departing proton will lower the mordant bath pH [49]. Alum was historically applied under mildly acidic conditions, often enabled by addition of cream of tartar (potassium hydrogen tartrate) [48], giving a mordant bath pH of 4-5. Under these mildly acidic conditions, side chains in the amino acids of wool and silk are deprotonated (aspartic acid pK_a 3.65, glutamic acid pK_a 4.25), allowing complexation between the positively charged metal cation and the negatively charge deprotonated amino acid side chain. When the dye is added to the bath the colorant molecules are able to displace water molecules in the metal coordination sphere and form a dye-mordant-fibre complex. Sometimes, post-mordanting was carried out with application of a different metal salt to bring about an improvement in wash fastness or a change in shade of the dyeing; typical examples of these mordants are hydrated iron sulphate ($FeSO_4 \cdot 7H_2O$) and tin chloride ($SnCl_2$) [2,48].

There is still debate about how the aluminium ions complex to the dye molecule. Kiel & Heertjes [50] first reported that the hydroxyl and the carbonyl functions in anthraquinones coordinate to the aluminium; it has been subsequently observed using ^{27}Al NMR that this proposed structure also exists in a dimeric form (Figure 2a) [51], however, ligands from the fibre will also contribute to the dye-mordant-fibre complex, so formation of a dimer is less likely. Theoretical studies have since suggested that coordination to the catechol moiety would be more favourable [52] (Figure 2b).

Figure 2

Disappointingly, there are not many conclusive crystal structures of these dye-metal complexes, and certainly none involving the fibre. Dyeing conditions during formation of the dye-mordant-fibre complex could also affect the metal complexes formed; for example, the –OH in the β-position to the carbonyl has a much higher pK_a value than the –OH in the γ-position due to an internal hydrogen bond with the adjacent carbonyl function [17]. Much of the research on metal complexes with dyes has been carried out with the aglycons alizarin and purpurin; however, it is also important to consider the coordination mechanisms of the glycosides in madder root as saccharide moieties possess many –OH functions able to act as electron donors to metals, hence it is likely that they are involved in complexation. Some theoretical work has been done on the coordination of alizarin glycoside with Ca^{2+}, which suggests that glycosylation increases binding affinity to the metal [53]. Stereochemistry is important in the ability of a saccharide moiety to coordinate to a metal ion; the sugar must have three adjacent hydroxyl groups in axial-equatorial-axial positions in chair form [54], and the terminal xylose and glucose of the primeveroside group present in both lucidin primeveroside [10] and ruberythric acid [11] have the ability to coordinate in this way. Little

experimental research has been done in considering the sugar moieties in anthraquinonoid colorants as the site of the molecule coordinating to the mordant metal, although some basic research exists on how sugars bind to a number of metals [54-56].

3.2. Extraction of colorants from fibres mordant-dyed with madder

The most commonly used literature extraction procedure uses a 37% hydrochloric acid: methanol: water (2:1:1, v/v/v) mixture [19,38,57-63]. In theory, this method works by H^+ ions in the strong acid displacing the Al^{3+} ions in the dye-mordant-fibre complex therefore releasing the dye into the solution [64].This method was developed by Wouters [19] and provides good yields of extracted dye, however, significant dye information is lost due to glycosidic bonds being hydrolysed and the destruction of the fibre [65]. Under the strongly acidic conditions used in this predominant literature method, most researchers concluded that alizarin was the primary dye component [19,38,57-59]; however, there is significant recent evidence that the presence of much of the alizarin in the analysed extract is as a result of ruberythric acid hydrolysis (**1**→**2**) [12,16,66,67], most likely via the glucoside, although isolation of the glucoside has not been reported. In addition, if the glycoside moieties of the anthraquinonoid colorants are coordinated to the aluminium, then the action of the acid breaking the glycoside bond will release the aglycon, leaving the sugar and metal complex intact.

When Wouters pioneered this technique over 30 years ago using HPLC he was unable to distinguish between certain extracts with similar elution times; without the additional analytical capability of chromatography-linked mass spectrometry, he identified alizarin and purpurin in 7th and 8th century Coptic textiles, but was unable to determine whether another peak was either pseudopurpurin or munjistin. It now seems clear from similar, recent work [67] that this was most probably munjistin, due to the instability of pseudopurpurin when extracted with HCl. The hydrochloric acid method has also been modified by adding a second extraction using 1:1 (v/v) MeOH: DMF to provide better extraction of most dyes [63]. This method can be problematic in that the high boiling point of DMF results in poor evaporation in a rotary evaporator and can hence get residual DMF traces in the products leading to dilution in the sample and reduced peak areas. There is also the same problem as above, in that strong acidic conditions cause degradation.

Different madder varieties and species with different origins have different chromatographic profiles in planta (Table 2), hence, the most effective artefact extraction technique would be one that preserves the colorants in the dyeings in the form as applied in the dyebath. Loss of information in the hydrolysis of glycoside-containing colorants into their aglycons means that resulting HPLC profiles cannot be matched to the corresponding plant dyestuffs [66]. Table 3 shows the possible assignment of Rubia species used to dye analysed artefacts based on anthraquinonoid colorants detected when extracted with 37% HCl: MeOH: water (2:1:1, v/v/v), which clearly demonstrates that

this technique is unhelpful in this assignment due to the significant damage caused to the colorants present.

To address the hydrolysis issue with 'hard' extraction techniques, recent research has focused on the development of 'softer' and effective extraction techniques to enable better-informed identification of the original dyestuff. This should be considered alongside known or anticipated reactivity of the molecules within the dye mixture. Soft extraction methods may also provide information about the dyeing process, for example, in 1860, Fabre patented [38] a process to produce "garancine", which involved intentionally breaking down the glycosides in madder by treatment with sulfuric acid before dyeing to obtain more free anthraquinones, which was believed to increase the efficiency of extraction of the dye components from the madder root.

Table 3. Possible assignment of Rubia species used to dye artefact based on anthraquinonoid colorants detected after extraction with 37% HCl: MeOH: water (2:1:1, v/v/v).

Compound detected	Rubia species possibly detected
alizarin (**2**)	R. tinctorum, R. cordifolia (trace), R. peregrina (trace)
nordamnacanthal (**5**)	R. tinctorum, R. peregrina, R. sikkimensis, R. yunnanensis
munjistin (**6**)	R. tinctorum, R. cordifolia, R. peregrina, R. sikkimensis, R. yunnanensis, R. akane
xanthopurpurin (**7**)	R. tinctorum, R. cordifolia, R. peregrina, R. sikkimensis, R. yunnanensis, R. akane (trace)
purpurin (**11**)	R. tinctorum, R. cordifolia, R. peregrina, R. sikkimensis, R. yunnanensis, R. akane
rubiadin (**13**)	R. tinctorum (trace), R. cordifolia (trace), R. peregrina, R. yunnanensis (trace)
6-hydroxyrubiadin (**29**)	R. cordifolia, R. sikkimensis, R. yunnanensis, R. akane

3.3. 'Soft' methods of extraction of colorants from fibres mordant-dyed with madder

Zhang & Laursen [66] developed two soft methods of extraction of silk mordant-dyed with madder. The first method involved extraction with formic acid: methanol (5:95, v/v) at 40 °C for 30 min; the second was the ethylenediaminetetraacetic acid (EDTA;) method which used H_2EDTA: acetonitrile: methanol (2:10:88, v/v/v) at 60 °C for 30 min. EDTA is a very strong chelating ligand with aluminium, therefore, it was proposed that it would complex to the aluminium with more affinity than the dye and hence release the dye from its insoluble complex into solution. Zhang & Laursen demonstrated that higher extraction yields were achieved with both the formic acid and EDTA methods, in comparison with the HCl: MeOH: water (2:1:1, v/v/v) method, and ventured that much more

information about the nature of the original dyestuff could be obtained using these soft methods because glycosidic dye components were not decomposed. Their initial tests on 1000-3000 year-old wool textile samples suggested that formic acid was more efficient than EDTA in extraction of flavonoid glycosides; they confirmed this with further work on analysis of yellow flavonoid glycosides used to dye silk artefacts [68].

Zhang et al. [67] applied the formic acid method to extract textile artefacts from a burial site in Chärchän, Xinjiang, China, which were dated at about 1000 BC, and found that red fragments contained the glycosides ruberythric acid, lucidin primeveroside, rubiadin primeveroside (unassigned by Zhang et al., but most probably this compound based on M_r detected and presence of rubiadin), as well as the aglycons alizarin, purpurin, rubiadin, munjistin, and xanthopurpurin; however, only the aglycons were detected when the same samples were extracted using the HCl method. This is interesting not only because it again highlights the damage caused by the HCl extraction process, but also because it demonstrates the potential stability of these anthraquinone glycosides, which were dyed over 3000 years ago. The authors did not identify the madder species used for the dyeing, but the evidence of ruberythric acid, lucidin primeveroside, and rubiadin primeveroside on analysis would point to *R. tinctorum*.

A variation on the EDTA method was extraction with 0.1% (aq.) disodium EDTA: DMF (1:1, v/v) [69]; this was shown to be non-destructive and almost completely conserved the fibre structure [65]. As with above the EDTA method, this works theoretically by the EDTA binding to the aluminium and displacing the dye molecules, but it is interesting to note that when EDTA was used in water alone or in water-methanol mixtures the dye was not released; the addition of DMF to the extraction solvent was required to enable dye released, but this caused a decrease in solubility of EDTA so only low concentrations (0.1%) could be used [69].

Valianou et al. developed extraction techniques using a 0.5 M aqueous solution of citric acid [70] and 2 M aqueous trifluoroacetic acid (TFA) solution [71] for extraction of flavonoid glycosides from yellow dyeings. The theory behind these techniques was that the weaker acids provided an extraction process with a less detrimental effect to acid-sensitive compounds and that it was also possible for these carboxylic acids to chelate to the mordant metal (Al^{3+}), which may aid extraction by disrupting the dye-metal complex as the –COOH functions are very good ligands for metal binding. The authors never demonstrated whether the citric acid technique was able extract anthraquinone glycosides. When extracting wool artefacts (1250-1517 AD) dyed with an unknown Rubia species with TFA, only aglycons, mainly alizarin and purpurin, were detected [70]. Recently, Ford et al. [16] showed that there was evidence that these two 'softer' techniques still had detrimental effects on wool dyed with *R. tinctorum*, wherein it was observed that lucidin primeveroside and ruberythric acid were detected in very low concentrations on extraction with citric acid and TFA, in

contrast to much higher concentrations observed in planta and when extracted by other soft techniques, suggesting that these glycosides are sensitive to acid hydrolysis even under mildly acidic conditions; the main compounds observed were the aglycons alizarin and purpurin.

Dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) have been employed to remove dyes from textile artefacts [70,72], but neither solvent has been demonstrated as able to remove mordanted dyes without an additional acid extraction step, nor have they shown an ability to remove anthraquinone glycosides intact. In addition, DMF sometimes gives extraneous adducts (+71 Da) of some anthraquinones, further complicating analysis. It likely that DMSO can oxidise lucidin to nordamnacanthal through a Kornblum/Swern type oxidation [73], hence, DMSO is probably not an appropriate solvent for use in madder extraction, particularly if used in combination with acid, which catalyses this type of oxidation. Han et al. [74] recently developed a two-step process whereby fibre dyed with R. cordifolia was initially extracted with DMSO and then subsequently extracted with an aqueous 0.5 M oxalic acid: water: acetone: methanol (1:30:40:30, v/v/v/v); the two fractions were then combined. Whilst it was found that the method could extract glycosides and aglycons (and reduce hydrolysis reactive in comparison with classic HCl techniques), it was not clear which step of the extraction was responsible for extraction of each identified component. In the chromatograms reported in the work an unidentified peak was seen at 27.6 min, which is most likely nordamnacanthal based on previous literature [12,16]. As no lucidin was observed in the extracts, despite the presence of lucidin primeveroside, it is likely that nordamnacanthal was produced by oxidation of lucidin by DMSO used in the extraction.

Mouri & Laursen [12] used an extraction solvent mixture of pyridine: water: 1.0 M (aq.) oxalic acid (95:95:10, v/v/v) on several Rubia species and demonstrated that this technique was capable of extracting glycosides, aglycons, and carboxylated aglycons. They proposed that this technique was capable of distinguishing between Rubia species based on HPLC analysis and stated that if 6-hydroxyrubiadin glycosides were observed that R. akane was most likely the source of the dye; however, as can be seen in Table 2, these compounds are also observed in significant concentrations in R. cordifolia and in the lesser-used R. sikkimensis and R. yunnanensis. They stated that R. tinctorum is distinctive because it is the only this species containing large amounts of alizarin and ruberythric acid, hence, whichever extraction method is used this conclusion can be drawn. They also noted that chay root (Oldenlandia umbellata), which grows in South India, contains only alizarin (i.e. no purpurin), enabling it to be distinguished from Rubia species when used for dyeing. However, they were unable to find marker pigments that could distinguish R. cordifolia and R. peregrina from each other.

Lombardi et al. [75] developed a soft alkaline extraction technique using 15% NH_3 (aq.): 1 mM disodium EDTA (1:1, v/v); this was compared to the EDTA method first developed by

Tiedemann & Yang [76] using 0.1% (aq.) Na_2EDTA: DMF (1:1, v/v). It was observed, on extraction of wool dyed with R. tinctorum, that both techniques removed ruberythric acid and lucidin primeveroside, and their respective aglycons, although the aglycons were extracted more efficiently by the ammonia method. Interestingly, both techniques also detected lucidin glucoside and alizarin glucoside, which have not been observed in planta, or in any previous extraction methods. It is likely that the solvent systems selectively hydrolysed the disaccharide to monosaccharide during extraction; it was observed by the authors that this effect was greater with DMF in comparison with ammonia.

More recently, Ford et al. [16] employed aqueous D-(+)-glucose solution (0.4% w/v) to extract wool samples dyed with the three R. tinctorum varieties; it was demonstrated on extraction of the dyed wool samples that the glycosides ruberythric acid and lucidin primeveroside were present in the R. tinctorum varieties they noted as 'Iranian madder' and 'Turkish madder', which were not observed in significant concentrations using HCl, citric acid or TFA extraction methods. It was suggested that this novel glucose method preserved anthraquinone glycosides on extraction and was also able to extract aglycons from the dyed fibre; alizarin, lucidin, purpurin, and rubiadin were extracted in very similar concentrations to those observed in planta. Other sugars (xylose, galactose, fructose, and sucrose) were also evaluated to see the efficiency of the extraction technique, and it was observed that although other sugars were able to extract glycosides efficiently, although the relative concentration of alizarin extracted was not as high as for glucose extractions.

The mechanism proposed was based on the nature of the hydroxyl groups in glucose and their ability to bind with and disrupt the dye-Al-fibre complex; glucose has the desirable axial-equatorial-axial stereochemistry required for optimal metal binding [54]. It was suggested that the glucose solution provides a favourable extraction medium as glucose can competitively bind to sugar moieties in the anthraquinone glycosides as it is capable of multiple hydrogen bonding interactions, thus displacing glycosylated dye into aqueous solution. The glucose extraction method was evidently able to overcome the binding forces operating between the mordant metal (Al^{3+}) and the dye as the method was effective in extracted aglycons also. They also demonstrated that the technique was equally effective for artificially aged wool dyeings.

Table 4 provides a comparison of the various 'hard' and 'soft' extraction techniques applied the madder-dyed textiles. It can be clearly seen that extraction with HCl, TFA and citric acid techniques does not preserve glycosylation. Other soft extraction techniques such as formic acid-methanol, pyridine-aqueous oxalic acid solution, EDTA-ammonia, and aqueous glucose solution are clearly able to preserve glycosylation in extraction of both reference dyed fibres and textile artefacts. This is an important observation as much literature states that the main compound present in artefacts dyed with R. tinctorum is alizarin and that any ruberythric acid present hydrolyses over many years of aging, yet is it clear from some soft extraction techniques that ruberythric acid is the often major

component extracted in both artefacts and artificially aged samples, in some cases where alizarin is not detected at all [12,16,67]. 6-hydroxyrubaidin glycosides are also able to be extracted from artefacts, most probably dyed with R. akane, using soft techniques [12].

Table 4. Compounds detected in madder dyed fibres using various literature extraction methods. + indicates compound detected in textile sample extract, tr. indicates trace amount detected. Fibre types indicated were dyed as part of the reference study, unless specified as an artefact (dates given).

Extraction method	Rubia species	Fibre	glycosides				aglycons								aglycon + carboxyl	
			1	3	12	24-28	2	4	5	7	11	13	20	29	6	10
HCl[a] [19]	R. tinctorum	Wool					+				+	+			+	
HCl[a] [19]	unknown Rubia spp.	7th and 8th C textiles					+				+				+	
HCl[a] [58]	unknown Rubia spp.	Burial textile (200-500 BC)					+				+					
HCl[a] [59]	unknown Rubia spp.	Wool artefact (206 BC-420 AD)					+				+					
HCl[a] [61]	R. tinctorum	Wool					+		+	+	+	+	+		+	
HCl[a] [62]	unknown Rubia spp.	Wool artefact (200-400 BC)					+			+	+					
HCl[a] [63]	unknown Rubia spp.	17th C costume					+				+					
HCl[a] [64]	unknown Rubia spp.	Burial textile (ca. 1000 BC)					+				+	+				
HCl[a] [67]	probably R. tinctorum	Burial textile (ca. 1000 BC)					+			+	+	+			+	
HCl[a] [74]	R. cordifolia	Silk					tr.				tr.	tr.		+		
Citric acid[b] [16]	R. tinctorum	Wool					+		+		+	+				
TFA[c] [16]	R. tinctorum	Wool					+	+			+	+				
TFA[c] [70]	unknown Rubia spp.	Wool artefact (1250-1517 AD)					+	+			+	+				
Formic acid[d] [67]	probably R. tinctorum	Burial textile (ca. 1000 BC)	+	+	+		+			+	+	+			+	
Pyr-OA[e] [12]	R. akane	Wool				+				+	+				+	+
Pyr-OA[e] [12]	R. tinctorum	Wool	+	+			+		+	+	+				+	+
Pyr-OA[e] [12]	R. cordifolia	Silk								+	+				+	+
Pyr-OA[e] [12]	R. peregrina	Wool								+	+				+	+
Pyr-OA[e] [12]	R. akane	Museum textile (ca. 700 AD)				+				+	+			+	+	+
Pyr-OA[e] [12]	R. tinctorum	Burial textile (ca. 1000 BC)	+	+												
DMSO-OA 2-step[f] [74]	R. cordifolia	Silk		tr.		+	tr.		+		+			tr.		
Na_2EDTA-DMF[g] [75]	R. tinctorum	Wool	+	+			+	+								
Na_2EDTA-NH_3[h] [75]	R. tinctorum	Wool	+	+			+	+								
Glucose[i] [16]	R. tinctorum	Wool	+	+			+	+	+		+	+				
Glucose[i] [16]	R. tinctorum	Wool (artificially aged)	+	+			+	+	+		+	+				

[a]37% (aq.) hydrochloric acid: methanol: water (2:1:1, v/v/v); [b]0.5 M (aq.) citric acid; [c]2 M (aq.) trifluoroacetic acid; [d]formic acid: methanol (5:95,v/v); [e]pyridine: water: 1.0 M (aq.) oxalic acid (95:95:10, v/v/v); [f](step 1) DMSO, (step 2) 0.5 M (aq.) oxalic acid: water: acetone: methanol (1:30:40:30, v/v/v/v); [g]0.1% (aq.) Na_2EDTA: DMF (1:1, v/v); [h]15% NH_3 (aq.): 1 mM Na_2EDTA (1:1, v/v); [i]0.4% (aq.) D-(+)-glucose.

However, it is not just hydrolysis of glycoside moieties that is caused by extraction using strong acids. Mouri & Laursen [12] observed that pseudopurpurin (**10**) is easily decarboxylated to

purpurin (**11**), and Derksen et al. [17] demonstrated that this occurs much more rapidly than the decarboxylation of munjistin (**6**) to xanthopurpurin (**7**). It is evident from Table 4 that munjistin is able to survive the HCl extraction method, but pseudopurpurin is not, it only being observed for certain Rubia species when extracted with pyridine-aqueous oxalic acid solution. Other soft extraction techniques may be able to preserve pseudopurpurin, but this has not been exemplified for relevant Rubia species. It is also particularly notable from Table 4 that no carboxylated glycosides (**8**, **9**, **31**) have been detected in extracted fibre samples, most probably due to their rapid deglycosylation by endogenous enzymes in the madder root [15].

Herein this review proposes the flowchart shown in Figure 3 as a decision tool to enable Rubia species to be distinguished from one another based on compounds detected in extraction and analysis of dyed fibre samples; this assumes that the extraction methods used are soft and preserve chemical structure integrity to enable a valid assessment, but does take into account very sensitive components such as galiosin (**8**) and pseudopurpurin (**10**).

Figure 3

3.4. Chromatographic techniques

For the majority of solvent extractions of madder-dyed textiles, during analysis the method of separation of the extract mixture is high performance liquid chromatography (HPLC) coupled with diode array detection (DAD) [77]. This technique gives high resolution separation of dyes involved and DAD produces peaks based on the UV-Vis spectral absorption properties of the chromophore of the colorants present. Chromatograms and UV-Vis data are compared to reference peaks and the compounds can be identified. Separation of dye components using HPLC techniques is usually made using a C18 reversed-phase column eluted with acetonitrile-water gradients containing 0.1% formic acid in both solvents. In literature, many different gradients and specific columns have been used, but elution times of the peaks for madder extracts were always in the same order; typical retention times observed for the various compounds extracted from madder can be grouped as seen in Table 5 based on their polarity [12].

More recently, HPLC has been paired with mass spectrometry (LC-MS) as the fragmentation patterns can provide further information on the structure of unknown compounds that are not found by DAD [66,78]. Research has shown that setting the mass spectrometer to negative ion mode is preferable for the detection of anthraquinone derivatives due to their polar nature [79]. Some older literature uses droplet counter current chromatography (DCCC) [17,66], but the mass of pure dye compounds recovered by these processes is in the magnitude of several milligrams; with this recovery being so low, limited analysis work has been performed on anthraquinone glycosides.

Table 5. Correlation between retention times (order of elution) and chemical structures of anthraquinone dye derivatives found in various Rubia species.

Retention times in terms of order of elution	Functionality (other than hydroxyl)	Compounds
first group	carboxylic acid + glycosyl	**8**, **9**, **31**
second group	glycosyl only	**1**, **3**, **12**, **24-28**
third group	carboxylic acid only	**6**, **10**
fourth group	no glycosyl or carboxylic acid	**2**, **4**, **5**, **7**, **11**, **13**, **20**, **29**

Recent research by Han et al. [74], using soft extraction techniques, for the first time applied Ultra High Performance Liquid Chromatography (UHPLC) coupled to both Photodiode Array detection (PDA) and Electrospray Ionisation Mass Spectrometry (ESI-MS) for chemical characterisation. Whilst the technique shows promise, it was unable to resolve completely major carboxylated components (munjistin and pseudopurpurin) typically observed in various Rubia species.

4. In situ techniques for analysis of textile artefacts dyed with different Rubia species

Techniques that do not involve extracting the dye from the sample for analysis, and therefore keep the sample intact, are non-destructive; the desire for such techniques from conservators is an obvious one as not have to surrender a piece of the artefact to be analysed is advantageous. These methods are generally useful for identifying the types of dye present, but typically not yield the level of detail afforded by the latest soft extraction techniques used with chromatography and mass spectrometry. As the in situ methods detect all of the dye molecules present, because there is no separation as achieved by chromatography, only the functional chemical groups present in the sample that are detected.

One method uses surface-enhanced Raman spectroscopy (SERS) for the identification of the dyestuff. Raman scattering is not usually considered for the detection of organic colorants as strong fluorescence from organic compounds masks the scattering pattern, however, in the case of SERS the fluorescence is quenched by a metal surface; silver nanoparticles are usually used as the metal surface as they can be easily reduced by citrate reduction or photo-reduction and can in some cases be applied directly to the sample [80-83]. Jurasekova et al. [81] demonstrated that this non-extractive, non-hydrolysing, in situ SERS method was capable of identifying flavonoids on mordant-dyed wool and

silk. SERS has also been coupled with Fourier-transform infrared spectroscopy (FT-SERS), which is reported to distinguish between the types of dye present based on the absorption peaks present from the main chromophore. However, if more than one dye is present there can be problems with overlapping spectra and results can be misleading, as has been shown for madder-dyed wool [84]. One advantageous application of SERS was found when Rambaldi et al. [85] used the method to analyse wool dyed with R. tinctorum; they found that significant concentrations of pseudopurpurin could be detected in the dyed sample, which was particularly noteworthy considering how unstable the carboxylated molecule is to extraction methods. The combination of excitation-emission matrix (EEM) fluorescence spectrometry and ultraviolet (UV-Vis) reflectance spectrometry can be used to distinguish between dye chromophores [86]; this is an attractive method as it does not require samples to be taken and is none destructive. However, similarly to FT-SERS, if there is more than one dye in the source being analysed the peaks can overlap due to the fact that the different chromophores cannot be separated.

Both time-of-flight secondary ion mass spectroscopy (TOF-SIMS) [87] and direct analysis in real time-time-of-flight mass spectrometry (DART-TOF-MS) [88] have been used to study purpurin and alizarin, silk and cotton dyed with these two anthraquinones, and historical textile samples. The mass spectra showed peaks relative to the molecular ions ($[M+H]^+$) and peaks relative to the characteristic fragmentation of each molecule, and purpurin and alizarin could be detected in analysed dyeings. However, there is no evidence of whether the technique can be applied to all dye components of madder, especially more sensitive structures. TOF-SIMS is useful in its ability to concurrently detect peaks relative to elemental ions from the mordant metal used.

Recently, a new method directly analysing the surface of different reddish-coloured historic textile samples was reported using flowprobe™-electrospray ionization-high-resolution mass spectrometry (flowprobe™-ESI-HRMS) [89]. The benefits of this real-time in situ microextraction method were reported as being rapid and reliable with minimal destruction of the sample. However, the detail provided by this analysis was even less precise than corresponding HPLC/LC-MS techniques; only aglycons could be detected and even then only general chemical structure detail was elucidated. In samples of red-coloured archaeological textiles from China and Peru, flowprobe™ HRMS detected: (i) dihydroxyanthraquinones; (ii) trihydroxyanthraquinones; (iii) dihydroxymethylanthraquinones; and (iv) dihydroxyanthraquinone carboxylic acids. However, comparative analysis by HPLC-DAD or HPLC-MS/MS, following extraction with HCl-MeOH, was additionally required to confirm the specific compounds as: (i) either alizarin or xanthopurpurin; (ii) purpurin; (iii) rubiadin; and (iv) a “munjistin-like compound”.

Although these methods can be used to identify different dye materials in samples without causing any damage to the artefacts they are not always useful as the different glycosides cannot be

seen using these techniques. The concentrations of the different colorants in madder cannot be quantified and the ratios of these concentrations cannot be compared. These methods can be useful for studies measuring the degradation of natural dyes when subjected to artificial ageing [90].

Conclusions

'Soft' extraction techniques are needed in the analysis of madder-dyed textiles due to the sensitive nature of these molecules, and their preservation up to the point of detection is key to unlocking full and valuable chemical information embedded in the dye structure, which otherwise is lost. Detailed analysis of dyes in textile artefacts can reveal important cultural and heritage information concerning historical textiles, providing evidence towards the specific dye species used, where this may have grown, how and where it was dyed, and perhaps where it was traded. Another key aspect is one of preservation, and the aim is to fully understand the nature of these dyestuffs in order to determine degradation patterns and structure-efficient conservation techniques. Understanding the precise molecular structure of these natural dyes and their chemical reactivity is important to provide knowledge of their interactions with physical substrates, such as textile fibres, which could be used to develop superior techniques for analysis of artefacts. The future of the detection of colorants in the study of artefacts could involve sequential application of different analysis methods where each technique could be used for maximum advantage. For example, an initial analysis of Rubia-dyed fibres with SERS to ascertain concentrations of pseudopurpurin (notably very unstable to extraction), followed by soft extraction and analysis by HPLC-DAD/LC-MS, could provide an all-encompassing method that provides maximum detection.

References

1. F Brunello, The Art of Dyeing in the History of Mankind (Vicenza: Neri Pozza, 1973).
2. J Hofenk de Graaff, The Colourful Past (London: Archetype Publications Ltd, 2004).
3. mpns.kew.org, accessed 18/04/2017.
4. P-J Robiquet, J-J Colin, J. Pharm. **12** (1826), 407.
5. J-J Colin, P-J Robiquet, Ann. Chim. Phys. **34** (1827), 225.
6. Rochleder, Justus Liebigs Ann. Chem. **80** (1851) 321.
7. G C H Derksen, Red, Redder, Madder – Analysis and Isolation of Anthraquinones from Madder Roots (Rubia tinctorum), Dissertation, (Wangeningen University, 2001).
8. I Boldizsár, Z Szűcs, Z Füzfai, I Molnár-Perl, J. Chromatogr. A **1133** (2006) 259.
9. G Cuoco, C Mathe, P Archier, F Chemat, C Vieillescazes, Ultrason. Sonochem. **16** (2009) 75.
10. R L Henderson, C M Rayner, R S Blackburn, Phytochemistry **95** (2013) 105.
11. L Ford, C M Rayner, R S Blackburn, Phytochemistry **117** (2015) 168.

12. C Mouri, R Laursen, Microchim. Acta **179** (2012) 105.
13. R Singh, Geetanjali, S M S Chauhan, Chem. Biodivers. **1** (2004) 1241.
14. R Wijnsma, R Verporte, In R A Hill, H C Krebs (Eds), Fortschritte der Chemie,Organischer Naturstoffe, Progress in the Chemistry of Organic Natural Products, Vol. 49 (Wein: Springer-Verlag, 1986).
15. R Hill, D Richter, Proc. Royal Soc. B **121** (1937) 547.
16. L Ford, R L Henderson, C M Rayner, R S Blackburn, J. Chromatogr. A **1487** (2017) 36.
17. G C H Derksen, H A G Niederlander, T . van Beek, J. Chromatogr. A **978** (2002) 119.
18. Y-S Han, R V der Heijden, R Verpoorte, Plant Cell Tiss. Org. **67** (2001) 201.
19. J Wouters, Studies Conserv. **30** (1985) 119.
20. U Özgen, C Kazaz, H Seçen, İ Çaliş, M Coşkun, P J Houghton, Turk. J. Chem. **33** (2009) 561.
21. G Cuoco, C Mathe, P Archier, C Vieillescazes, J. Cult. Herit. **12** (2011) 98.
22. M-J Liou, P-L Wu, T-S Wu, Chem. Pharm. Bull. **50** (2002) 276.
23. Y Yasui, N Takeda, Mutat. Res. **121** (1983) 185.
24. A M Tessier, P Delaveau, B Champion, Planta Med. **41** (1981) 337.
25. H Itokawa, Y Qiao, K Takeya, Phytochemistry **28** (1989) 3465.
26. N R Ayyangar, K Venkataraman, J. Sci. Indian. Res. **15B** (1956) 359.
27. Y Kawasaki, Y Goda, K Yoshihira, Chem. Pharm. Bull. **40** (1992) 1504.
28. J Koyama, T Ogura, K Tagahara, T Konoshima, M Kozuka, Phytochemistry **31** (1992) 2907.
29. J-T Fan, B Kuang, G-Z Zeng, S-M Zhao, C-J Ji, Y-M Zhang, N-H Tan, J. Nat. Prod. **74** (2011) 2069.
30. M Wada, Science **11** (1941) 415.
31. S X Wang, H M Hua, L J Wu, X Li, T R Zhu, Acta Pharm. Sin. **27** (1992) 743.
32. E Y Backheet, S F Farag, N A El-Emary, Bull. Faculty Pharm. **39** (2001) 135.
33. H Itokawa, Z Z Ibraheim, Y F Qiao, K Takeya, Chem. Pharm. Bull. **41** (1993) 1869.
34. A M Vidal-Tessier, P Delaveau, B Champion, Ann. Pharm. Fr. **45** (1987) 261.
35. Z Z Ibraheim, Bull. Pharm. Sci. **25** (2002) 85.
36. E Schunck, L Marchlewski, J. Chem. Soc., **63** (1893) 969; J. Chem. Soc. **65** (1894) 182.
37. A Vaidyanathan, Dyes Pigm. **6** (1985) 27.
38. J Wouters, Dyes Hist. Archaeol. **10** (1992) 17.
39. E S B Ferreira, A N Hulme, H McNab, A Quye, Chem. Soc. Rev. **33** (2004) 329.
40. E Schunck, J. Prakt. Chem. **63** (1854) 222.
41. S Yamazaki, In Y Orimono, M Kimura, S Yamazaki (Eds), The Reproduction of Noh Costumes (Tokyo: Traditional Cultures Exchange Forum, 1984).

42. J Hellot, *L'A*rt de la teinture des laines et des étoffes de laine en grand et petit teint avec une instruction sur les débouillis (Paris: Veuve Pissot, 1750).
43. T Aburjai, F M Natsheh, Phytotherapy Res. **17** (2003) 987.
44. G C H Derksen, M Naayer, T A van Beek, A Capelle, I K Haaksman, H A van Doren, A E de Groot, Phytochem. Anal. **14** (2003) 137.
45. H Schweppe, Handbuch der Naturfarbstoffe, (Landsberg/Lech: Ecomed Verlagsgesellschaft, 1993).
46. G Derksen, T A van Beek, A E de Groot, A Capelle, J. Chromatogr. A **816** (1998) 277.
47. P Novotna, V Pacakova, Z Bosakova, K Stulik, J. Chromatogr. A **863** (1999) 235.
48. D Cardon, Natural Dyes, Sources, Tradition, Technology and Science, (London: Archetype, 2007), pp. 107-166.
49. D F Shriver, P W Atkins, Inorganic Chemistry, (3rd edition, New York: Oxford University Press, 2002).
50. E G Keil, P M Heertjes, J. Soc. Dyers Colour. **79** (1963) 21.
51. P Soubayrol, G Dana, Magn. Reson. Chem. **34** (1996) 638.
52. Y Di Iorio, R Parra, K Szaciłowski, M A Grela, New J. Chem. **37** (2013) 969.
53. D Toczek, K Kubas, M Turek, S Roszak, R Gancarz, J. Mol. Model. **19** (2013) 4209.
54. L Nagy, A Szorcsik, J. Inorg. Biochem. **89** (2002) 1.
55. B Gyurcsik, L Nagy, Coordin. Chem. Rev. **203** (2000) 81.
56. M Bera, A Patra, A. Carbohydr. Res. **346** (2011) 2075.
57. I Karapanagiotis, S Daniilia, A Tsakalof, Y Chryssoulakis, J. Liquid Chromatogr. Rel. Technol. **28** (2005) 739.
58. G Balakina, V Vasiliev, E Karpova, V Mamatyuk, Dyes Pigm. **71** (2006) 54.
59. J Liu, D Guo, Y Zhou, Z Wu, W Li, F Zhao, X Zheng, J. Archaeol. Sci. **38** (2011) 1763.
60. L Rafaëlly, S Héron, W Nowik, A Tchapla, Dyes Pigm. **77** (2008) 191.
61. I Surowiec, B Szostek, M Trojanowicz, J. Sep. Sci. **30** (2007) 2070.
62. A Kramell, X Li, R Csuk, M Wagner, T Goslar, P E Tarasov, N Kreusel, R Kluge, C-H Wunderlich, Quat. Int. **348** (2014) 214.
63. I Surowiec, A Quye, M Trojanowicz, J. Chromatogr. A **1112** (2006) 209.
64. J Sanyova, J Reisse, J. Cult. Herit. **7** (2006) 229.
65. A Manhita, T Ferreira, A Candeias, CB Dias, Anal. Bioanal. Chem. **400** (2011) 1501.
66. X Zhang, R Laursen, J. Anal. Chem. **77** (2005) 2022.
67. X Zhang, I Good, R Laursen, J. Archaeol. Sci. **35** (2008) 1095.
68. X Zhang, R Boytner, J L Cabrera, R Laursen, Anal. Chem. **79** (2007) 1575.
69. E J Tiedemann, Y Yang, J. Am. Inst. Conserv. **34** (1995) 195.

70. L Valianou, I Karapanagiotis, Y Chryssoulakis, Anal. Bioanal. Chem. **395** (2009) 2175.
71. D Mantzouris, I Karapanagiotis, L Valianou, C Panayiotou, Anal. Bioanal. Chem. **399** (2011) 3065.
72. I Joosten, M R van Bommel, Microchim. Acta **162** (2008) 433.
73. K Omura, D Swern, Tetrahedron **34** (1978) 1651.
74. J Han, J Wanrooij, M van Bommel, A Quye, J. Chromatogr. A **1479** (2017) 87.
75. L Lombardi, I Serafini, M Guiso, F Sciubba, A Bianco, Microchem. J. **126** (2016) 373.
76. E J Tiedemann, Y Yang, J. Am. Inst. Conserv. **34**, 1995, 195.
77. G C Derksen, G P Lelyveld, T A van Beek, A Capelle, A E de Groot, Phytochem. Anal. **15** (2004) 397.
78. L Rafaëlly, S Héron, W Nowik, A Tchapla, Dyes Pigm. **77** (2008) 191.
79. J Rauha, H Vuorela, R Kostiainen, J. Mass Spectrom. **36** (2001) 1269.
80. M Leona, J Stenger, E Ferloni, J. Raman Spectrosc. **37** (2006) 981.
81. Z Jurasekova, C Domingo, J V Garcia-Ramos, S Sánchez-Cortés, J. Raman Spectrosc. **39** (2008) 1309.
82. F Pozzi, J R Lombardi, S Bruni, M Leona, Anal. Chem. **84** (2012) 3751.
83. A Idone, M Gulmini, A-I Henry, F Casadio, L Chang, L Appolonia, R P Van Duyne, N C Shah, Analyst **138** (2013) 5895.
84. C Zaffino, S Bruni, V Guglielmi, E De Luca, J. Raman Spectrosc. **45** (2014) 211.
85. D C Rambaldi, F Pozzi, N Shibayama, M Leona, F D Preusser, J. Raman Spectrosc. **46** (2015) 1073.
86. R Nakamura, Y Tanaka, A Ogata, M Naruse, Anal. Chem. **81** (2009) 5691.
87. Y Lee, J Lee, Y Kim, S Choi, S W Ham, K-J Kim, Appl. Surf. Sci. **255** (2008) 1033.
88. C S DeRoo, R A Armitage, Anal. Chem. **83** (2011) 6924.
89. A E Kramell, A O Brachmann, R Kluge, J Piel, R Csuk, RSC Adv. **7** (2017) 12990.
90. M Koperska, T Łojewski, J Łojewska, Anal. Bioanal. Chem. **399** (2011) 3271.

Figure Captions

Figure 1. Possible inter-relationships between anthraquinone compounds found in Rubia species based on chemical or biochemical interconversion.

Figure 2. Dimeric metal complex of alizarin with Al^{3+} formed through coordination complex through: (a) 1-hydroxyl and the carbonyl functions; (b) catechol moiety.

Figure 3. Decision flowchart to enable Rubia species to be distinguished based on compounds extracted and analysed in dyed fibre samples. For compound number assignments see Table 2.