High performance liquid chromatography of some natural dyes: 
Analysis of plant extracts and dyed textiles

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High performance liquid chromatography of some natural dyes: Analysis of plant extracts and dyed textiles

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Abstract

In this study, wool fibre samples were mordanted by means of 25% \( \text{KAi(SO}_4\text{)}_{2.12\text{H}\text{O}} \) (alum) mordant solution. The mordanted wool samples were dyed in 50% \textit{Reseda luteola} L. (weld), 20% \textit{Rhamnus petiolaris} Boiss (buckthorn), and 50% \textit{Datisca cannabina} L. (bastard) hemp dye bathes. A reversed phase high performance liquid chromatography (RP-HPLC) with diode-array detection (DAD) method was utilized for the identification of dyes in the dyed wool samples and the plant extracts. The extraction of dyes was carried out with an \( \text{HCl/MeOH/H}_2\text{O} \) (2:1:1; \( \text{v/\text{v/v}} \)) mixture.

Introduction

Natural dyes are obtained from some plant and animals in nature [1-9]. They were the primary colour source of textiles until the mid - to late 19th century [10-13]. Flavonoids (flavones and flavonols) are the main chromophores in the most extensively used yellow dyes [14]. Another class of flavonoid contains many subgroups like flavanone, flavanonol, chalcone, aurone, neoflavonoids and biflavonoids etc. [15]. For a long time, natural dyes have been used for purposes such as the colouring of wool, cotton and silk natural fibers as well as fur and leather [10,11,16-18]. Historically, textiles were dyed with natural dyes, mainly of plant or insect origin, descriptions of which can be found in many sources, for example in books of Schweppe [19], Cardon [20], Hofenk-de Graaff [21], and Karadag [22].

The identification of natural dyes is very important in the scientific examination of the biological dye sources of historical textiles, paintings, illuminated manuscripts, coloured prints, conservation and restoration works [3,10,23]. Several articles based on the analysis of dyes in the natural dye sources and in the textiles have been published by various authors.
The predominant flavonoid components containing *Reseda luteola* were detected in 1992 by Wouters and Chirinos as luteolin and apigenin (flavones), at a ratio of approximately 9/1, measured at 255 nm [6]. In 1993 Kaiser reported quantitative analysis of flavonoid content in different organs of *Reseda luteola* L. [24]. Additionally, in 2003 Cristea *et al.* have achieved quantitative HPLC analysis of the main flavonoid compounds present in weld (*Reseda luteola* L.) [11]. In same year Orska-Gawryś *et al.* [25] employed reverse-phase HPLC with diode-array UV–Vis spectrophotometric detection for the identification of natural dyes in the extracts from wool and silk fibers from archeological textiles. Moreover, a minor flavonoid component from extracts of textile samples dyed with weld (*Reseda luteola* L.) was determined by HPLC-PDA in 2008 Peggie *et al.* [26]: chrysoeriol (3’-O-methyl-luteolin).

Similarly, in 2009 Marques *et al.* reported on the analysis of natural dyes in two seventeenth century Arraiolos carpets from the National Museum of Machado de Castro where high-performance liquid chromatography with UV–Vis diode array detection (HPLC–DAD) and HPLC–mass spectrometry (LC–MS) were utilized in the analyses [27]. In the same year, Samanta and Agarwal published a review article about application of natural dyes on textiles [28].

Woelfle *et al.*, on the other hand, reported on the determination of luteolin-7-O-glucoside, luteolin and apigenin as the main dyeing component in *Reseda luteola* plant. Moreover, minor components were identified as luteolin-3’,7-diglucoside, chrysoeriol-7-O-glucoside, luteolin-3’-O-glucoside, and luteolin-3’-methylether [29].

Weld appears to have been used in Europe since prehistoric times. It is likely to have been widely used in the Mediterranean area in the Hellenistic period and in the Roman Empire [14] era. *Reseda luteola* L. was used in the 1st century Masada textiles,
3rd century Palmyra textiles, 13th century Seljuk carpets and 15-20th century Ottoman textiles for yellow and green colours [22]. Another interesting plant is Bastard hemp (*Datisca cannabina* L.); it is very rich in flavonols: datiscetin (CI 75630) present in the form of a rutinoside, datiscin (amounting to 10% of the weight of the fresh leaves) and as three glucoside. Kaempferol, quercetin and galangin are also present. It is also the source of CI Natural Yellow 12. Dye analysis demonstrated that bastard hemp was used in carpets and kilims by Turkish dyers in the Balikesir regions of western Anatolia.

The use of buckthorn berries, on the other hand, was specially common in Turkey where *cehri* (or altın ağacı) (*R. petiolaris*) was cultivated in orchards. This plant was identified by Böhmer, in the course of his extensive examination and analysis of the colorants used in Turkish carpets, as being the source (in combination with madder) of the orangey-red colour used in the border of a beautiful 15th century prayer rug, which is now present in the Türk ve İslam Eserleri Müzesi in İstanbul [14]. This dye source was also used in the Turkish carpets from the 15th to 17th centuries [30].

In 1971 Tanker and Ertan published a paper about the chemistry of the anthraquinone derivatives in the bark of *Rhamnus petiolaris* [31]. A new acylated flavonol glycoside from the berries of *Rhamnus petiolaris* was isolated in 1994 by Çalış et al. [32]: rhamnetin 3-O-[3‴-O—(p-coumaroyl)-α-L-rhamnopyranosyl (1→3)- α -L-rhamnopyranosyl(1→6)]-β-D-galactopyranoside. Recently, we have identified natural dye components (rhamnetin and emodin) in natural pigments obtained from the extracts of berries of *Rhamnus petiolaris* plant [33].

The aim of the present paper is to qualitatively determine the natural dyes and natural dye components present in the dyed wool samples and in the dye plants by a reversed – phase high performance liquid chromatography (HPLC) with diode-array
detection (DAD) to shed some light about the plants investigated in this work.

Moreover, analysis of dyes extracted from textiles of historical interest can give valuable information as to where, when, and how the textiles were made. Additionally, this work, using three yellow dye plants: weld (Reseda luteola L.), buckthorn (Rhamnus petiolaris Boiss), and bastard hemp (Datisca cannabina L.) which belong to the natural dye source, focuses on dyeing protein fibers that can be an important advantage for the environmental processes. The dye extraction (mainly flavonoid and minor anthraquinone) from the dyed wool samples and the dye plants was performed by using previously described procedures [34-36].

Experimental
Materials

100 % wool yarns made of Z twill were used in the experimental study. The yarn count was Nm 4 and the twist of the wool yarn was 133 T/m. All reagents employed in this investigation were of analytical grade and were used as received, unless stated otherwise. High purity water was obtained by passing water though a Milli-Q treatment system (Millipore, Bedford, MA, USA) and the HPLC mobile phase was prepared using Milli-Q water. Weld (Reseda luteola L.), buckthorn (Rhamnus petiolaris Boiss), and bastard hemp (Datisca cannabina L.) were obtained from the Turkish Cultural Foundation, Research and Development Laboratory for Natural Dyes, Istanbul, Turkey. The following dye standards were used as references: luteolin (5,7,3',4'-tetrahydroxyflavone), rhamnetin (3,5,3',4'-tetrahydroxy-7-methoxy-flavone), isorhamnetin (3,5,7,4'-tetrahydroxy-3'-methoxyflavone), kaempferol (3,5,7,4'-tetrahydroxyflavone), emodin (1,3,8,trihydroxy-6-methylanthraquinone), apigenin (5,7,4'-trihydroxyflavone), datiscetin (3,5,7,2'-tetrahydroxyflavone), quercetin-3-
arabinosid and luteolin-7-glucoside from Carl Roth (Karlsruhe, Germany). Alum (KAl(SO$_4$)$_2$.12H$_2$O), hydrochloric acid, and methyl alcohol were purchased from Merck (Darmstadt, Germany, www.merck.de).

Mordanting

Mordanting was accomplished by using 25 % of mordant to textile (w/w) and carried out using the pre-mordanting method. This was performed at 100 °C for 1 h in a liquor ratio 40:1. Wool samples were then squeezed and dried at room temperature for four days.

Dyeing

Dyeing was performed by using 20 % buckthorn (dried berries), 50 % bastard hemp (stem), and 50 % weld (aboveground parts) dye plants to textile (w/w). First, the dye plants were seperately soaked in 200 ml of deionised boiled water. The mordanted wool samples were heated in a dye bath ratio of 100:1 at 100 °C for 30 min. Then the wool samples were removed from the dye bathes, rinsed with deionised water and dried at room temperature.

Methods

HPLC analysis

Chromatographic separations were carried out using an Agilent 1200 series system (Agilent Technologies, Hewlett-Packard, Germany) including a G1329A ALS autosampler, a G1315A diode-array detector. A G1322A vacuum degasser and a G1316A thermostatted column compartment were used. Chromatograms were obtained by scanning the sample from 191 nm to 799 nm with a resolution of 2 nm; eluted peaks were monitored at 255 nm and 350 nm. Data were analyzed using Agilent Chemstation.
A Nova-Pak C\textsubscript{18} analytical column (3.9 mm x 150 mm, 4 µm particle size; Part No. WAT 086344, Waters) protected by a guard column filled with the same material was used. Analytical and guard columns were maintained at 30°C. The HPLC gradient elution was performed using previously described methods [37,38]. Chromatographic separations of the hydrolysed samples were performed using a gradient elution program that utilizes two solvents: solvent A: H\textsubscript{2}O - 0.1% TFA (trifluoroacetic acid) and solvent B: CH\textsubscript{3}CN (acetonitrile) - 0.1% TFA. The flow rate was 0.5 ml/min and the elution program was as described earlier [37,38].

**Extraction procedure for HPLC analysis**

The extraction from the dye plants and the dyed wool samples was done as described in earlier reports [34-36]. The samples were prepared as follows:

The dye extraction from the dye plants was performed by using procedures 1 and 2.

1) In the first procedure the dye extraction from the dye plants (2.4-10.2 mg) were achieved in 400 µl of the mixture of MeOH: H\textsubscript{2}O (2:1; v/v) in a conical glass tube without heating (non-acid hydrolysed).

2) In the second procedure, organic dyes were extracted from the plants (2.5-9.0 mg) and the dyed wool samples (2.3-18.8 mg) were hydrolysed by heating in 400 µl of H\textsubscript{2}O:MeOH:37% HCl (1:1:2; v/v/v; 400 µl) in conical glass tubes for 8 min in a water-bath at 100 °C. After rapid cooling under running cold water, the solution was evaporated just to dryness in a water-bath at 50-65 °C under a gentle stream of nitrogen. The dry residues were dissolved in 400 µl of the mixture of MeOH: H\textsubscript{2}O (2:1; v/v). All mixtures were filtered using polypropylene syringe filters (0.2 µm) followed by injection of 10 and/or 100 µl of the supernatant were into the HPLC apparatus.
Results and discussions

In the present study, the wool samples dyed by the various yellow dye plants (Reseda luteola L., Rhamnus petiolaris Boiss, and Datisca cannabina L.) were analyzed qualitatively by a reversed phase high performance liquid chromatography (RP-HPLC) with diode-array detection (DAD). The dye extractions for HPLC analysis were done by using published procedures [37,38]. The sample preparation for the extraction of dye components from the dyed wool fibres is based on the commonly used hydrolysis procedure with hydrochloric acid. For the extracts, this treatment is necessary to isolate the organic dye from its mordant metal.

Weld (Reseda luteola L.)

In the acid hydrolysed weld and weld-dyed wool sample extracts, luteolin is observed together with relatively minor amounts of apigenin and a component identified as a chrysoeriol by HPLC-DAD (monitored at 255 and 350 nm). The main ingredients of the yellow dye source weld (R. luteola L.) are well documented; luteolin [25,39] is the major flavonoid component observed in a methanol/water extract, present as the parent compound and as its 7-O-glucoside, 3',7-di-O-glucoside [39] and chrysoeriol [40]. For all weld extracts, HPLC analysis showed the presence of three main flavonoid compounds. The bibliographical data, the UV/VIS spectra, and the retention times permitted the identification of these flavonoids: luteolin, apigenin [25], and chrysoeriol. Although chrysoeriol in the methanolic extract of R. luteola was not detected in a previously described report by Moiteiro et al. [13], but it could be detected here.
Buckthorn (*Rhamnus petiolaris* Boiss)

In sample derived from the non-hydrolysed *R. petiolaris* Boiss extract, the first eluted peak, shown as qu-3-arab in Fig. 2(a), was identified by comparison with pure quercetin-3-arabinosid compound. The other three peaks were: quercetin glucosides: qu-gluc-1 ($\lambda_{\text{max}}$: 256, 349), qu-gluc-2 ($\lambda_{\text{max}}$: 257, 267, 295, 356), and qu-gluc-3 ($\lambda_{\text{max}}$: 255, 267, 297, 355). The HPLC-DAD chromatogram (monitored at 255 nm) obtained from the acid hydrolysed buckthorn extract is shown in Fig. 2(b). The components were identified as structurally related flavonoids (rhamnetin and isorhamnetin) and anthraquinone (emodin) by comparison of their retention times and UV-visible spectra to either an in house library of pure standard compounds or with previously published data [33]. The main colouring components related to the hydrolysis plant extract were identified as isorhamnetin and rhamnetin, with a minor amount of emodin also present.

Fig. 2(c) shows the HPLC chromatogram collected for the sample extracted from the buckthorn-dyed wool fibres. Chromatographic identification of unknown dyes present in the acid hydrolysed wool extract requires initial determination of retention times of analytes based on available standard dye compounds. The absence of emodin has been reported in the non hydrolysed and acid hydrolysed *R. petiolaris* Boiss (buckthorn) in contrast to the acid hydrolysed buckthorn-dyed wool extract, in which emodin is the minor coloring component.

Bastard hemp (*Datisca cannabina* L.)

The HPLC-DAD chromatograms related to the acid hydrolysed *D. cannabina* L. extract and the dyed-wool extract are shown in Fig.3(b,c). Datiscetin was identified as a main component both in the acid hydrolysed *D. cannabina* L. extract and in the dyed-wool extract.
Contrary to datiscetin compound which is detected in these extracts, the main peak was detected in the HPLC-DAD analysis of sample prepared (Fig.3a) with a solution of methanol/water (2:1) mixture. Campos and Markham mentioned in their book that the spectrum investigation of various existed natural compounds seems to be in harmony with the spectrum of the main peak detected component ($\lambda_{\text{max}}$: 257, 305, and 329) datiscetin-3-O-[rhamnosyl(1-6)glucoside] [41].

Conclusions

Results presented in this study on the flavonoid (mainly) and anthraquinone (scarce) contents of $R$. luteola, $D$. cannabina, and $R$. petiolaris provided valuable information in terms of the development of new alternative crops such as dyeing plants. Aqueous extracts of the dye plants ($Rhamnus petiolaris$ Boiss, $Datisca cannabina$ L. and $Reseda luteola$ L.) could be used for dyeing wool fibres with mordanting by means of alum mordant. In addition, an analytical method is developed for the identification of dyes present in the wool samples dyed via the natural dye sources. The outcome of this study is that it would be possible to analyze and to identify the natural dyes present in historical textiles using a simple method.

Acknowledgement

The support by Turkish Cultural Foundation is gratefully acknowledged (www.turkishculturalfoundation.org).
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30. H Böhmer, Koekboya-Natural dyes and textiles (Ganderkesee-Germany: Remhöb-Verlag, 2002).
41. M G Campos and K R Markham, Structure information from HPLC and on-line measured absorption spectra: flavones, flavonols and phenolic acids (Março: Imprensa da Universidade de coimbra, 2007).
Table 1. Chromatographic and spectral characteristics of the investigated sample extracts.

<table>
<thead>
<tr>
<th>Dye (Standard)</th>
<th>Abbreviation</th>
<th>Retention time in the given protocol (min)</th>
<th>UV-VIS data</th>
</tr>
</thead>
<tbody>
<tr>
<td>datiscetin-3-O-[rhamnosyl (1-6)glucide]</td>
<td>da-3-O-[rham(1-6)gluc]</td>
<td>18.0</td>
<td>259, 305, 331</td>
</tr>
<tr>
<td>luteolin-3',7-di-O-glucoside</td>
<td>lu-3',7-di-O-gluc</td>
<td>18.4</td>
<td>240, 268, 341</td>
</tr>
<tr>
<td>quercetin-3-arabinosid</td>
<td>qu-3-arab</td>
<td>19.4</td>
<td>255, 267, 295, 355</td>
</tr>
<tr>
<td>luteolin-7-O-glucoside</td>
<td>lu-7-O-gluc</td>
<td>19.9</td>
<td>254, 267, 349</td>
</tr>
<tr>
<td>luteolin</td>
<td>lu</td>
<td>25.5</td>
<td>253, 267, 289, 347</td>
</tr>
<tr>
<td>datiscetin</td>
<td>da</td>
<td>26.4</td>
<td>257, 305, 347</td>
</tr>
<tr>
<td>apigenin</td>
<td>ap</td>
<td>26.5</td>
<td>265, 293, 337</td>
</tr>
<tr>
<td>isorhamnetin</td>
<td>isorhm</td>
<td>26.9</td>
<td>255, 301, 369</td>
</tr>
<tr>
<td>chrysoeriol</td>
<td>chr</td>
<td>27.1</td>
<td>250, 268, 289, 346</td>
</tr>
<tr>
<td>rhhamnetin</td>
<td>rhm</td>
<td>28.1</td>
<td>255, 299, 371</td>
</tr>
<tr>
<td>emodin</td>
<td>em</td>
<td>31.7</td>
<td>253, 267, 289, 439</td>
</tr>
</tbody>
</table>

Table 1. Chromatographic and spectral characteristics of the investigated sample extracts.

168x164mm (300 x 300 DPI)
Table 2. Identified dyes in the sample extracts.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dyes detected</th>
<th>Plant Non-hydrolysed extract</th>
<th>Plant Acid hydrolysed extract</th>
<th>Wool (Acid hydrolysed extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weld</td>
<td>Luteolin-3',7-di-O-glucoside</td>
<td>Luteolin</td>
<td>Luteolin</td>
<td>Luteolin</td>
</tr>
<tr>
<td></td>
<td>Luteolin-7-O-glucoside</td>
<td>Apigenin</td>
<td>Apigenin</td>
<td>Apigenin</td>
</tr>
<tr>
<td></td>
<td>Luteolin</td>
<td>Chrysoeriol</td>
<td>Chrysoeriol</td>
<td>Chrysoeriol</td>
</tr>
<tr>
<td>Buckthorn</td>
<td>Quercetin-3-arabinosid</td>
<td>Isorhamnetin</td>
<td>Isorhamnetin</td>
<td>Rhamnetin</td>
</tr>
<tr>
<td></td>
<td>Possible quercetin glucosides</td>
<td>Rhamnetin</td>
<td>Rhamnetin</td>
<td></td>
</tr>
<tr>
<td>Hemp</td>
<td>Datis cetin</td>
<td>Datis cetin</td>
<td>Datis cetin</td>
<td>Datis cetin</td>
</tr>
<tr>
<td></td>
<td>Datis cetin-3-O-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[rhamnosyl(1-6)glucoside]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Identified dyes in the sample extracts.
172x147mm (300 x 300 DPI)
Figure 1. Chromatograms of non-hydrolysed weld extract (a), acid hydrolysed weld extract (b), and acid hydrolysed weld-dyed wool extract (c).

Figure 1. Chromatograms of non-hydrolysed weld extracts (a), acid hydrolysed weld extract (b), and acid hydrolysed weld-dyed wool extract (c).

153x241mm (300 x 300 DPI)
Figure 2. Chromatograms of non-hydrolysed buckthorn extract (a), acid hydrolysed buckthorn extract (b), and acid hydrolysed buckthorn-dyed wool extract (c).
Figure 3. Chromatograms of non-hydrolysed bastard hemp extract (a), acid hydrolysed bastard hemp extract (b), and acid hydrolysed bastard hemp-dyed wool extract (c).