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

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In this study, wool fibre samples were mordanted by means of 25% alum mordant solution. The mordanted wool samples were dyed in 50% *Reseda luteola* L. (weld), 20% *Rhamnus petiolaris* Boiss (buckthorn) and 50% *Datisca cannabina* L. (bastard hemp) dyebaths. A reverse-phase high-performance liquid chromatography with diode-array detection method was utilised for the identification of dyes in the dyed wool samples and the plant extracts. The extraction of dyes was carried out with a hydrogen chloride/methanol/water (2:1:1; v/v/v) mixture.

Introduction

Natural dyes are obtained from some plants and animals in nature [1–9] and, until the mid- to late-nineteenth century, they were the primary colour source for textiles [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, such as flavanone, flavanonol, chalcone, aurone, neoflavonoids and biflavonoids, etc. [15]. For many years, natural dyes have been used for purposes such as the colouring of wool, cotton and silk natural fibres, as well as fur and leather [10,11,16–18]. Historically, textiles were dyed with natural dyes, mainly of plant or insect origin, and descriptions of these can be found in many sources; for example, in books by 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 their natural dye sources and in textiles have been published by various authors.

The predominant flavonoid components contained in *Reseda luteola* were detected in 1992 by Wouters and Chirinos [6] as luteolin and apigenin (flavones), at a ratio of approximately 9:1, measured at 255 nm. In 1993, Kaiser [24] reported quantitative analysis of flavonoid content in different organs of *Reseda luteola* L. Additionally, in 2003, Cristea *et al.* [11] achieved quantitative high-performance liquid chromatography (HPLC) analysis of the main flavonoid compounds present in weld (*Reseda luteola* L.). In the same year, Orska-Gawryś *et al.* [25] employed reverse-phase HPLC with diode-array ultraviolet–visible (UV–vis) spectrophotometric detection for the identification of natural dyes in the extracts from wool and silk fibres from archeological textiles. Moreover, a minor flavonoid component from extracts of textile samples dyed with weld (*Reseda luteola* L.) was determined by HPLC–photodiode array (PDA) in 2008 by Peggie *et al.* [10]: chrysoeriol (3′-*O*-metylluteolin).

Similarly, in 2009 Marques *et al.* [26] reported on the analysis of natural dyes in two seventeenth-century Arraiolos carpets from the National Museum of Machado de Castro where HPLC with UV–vis diode array detection (HPLC-DAD) and HPLC–mass spectrometry (HPLC-MS) were utilised in the analyses. In the same year, Samanta and Agarwal [27] published a review article about the application of natural dyes on textiles.

Woelfle *et al.*, in contrast, reported on the determination of luteolin-7-*O*-glucoside, luteolin and apigenin as the main dyeing component in the *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 [28].

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 era of the Roman Empire [14]. *Reseda luteola* L. was used in the first-century Masada textiles, third-century Palmyra textiles, thirteenth-century Seljuk carpets and fifteenth- to twentieth-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

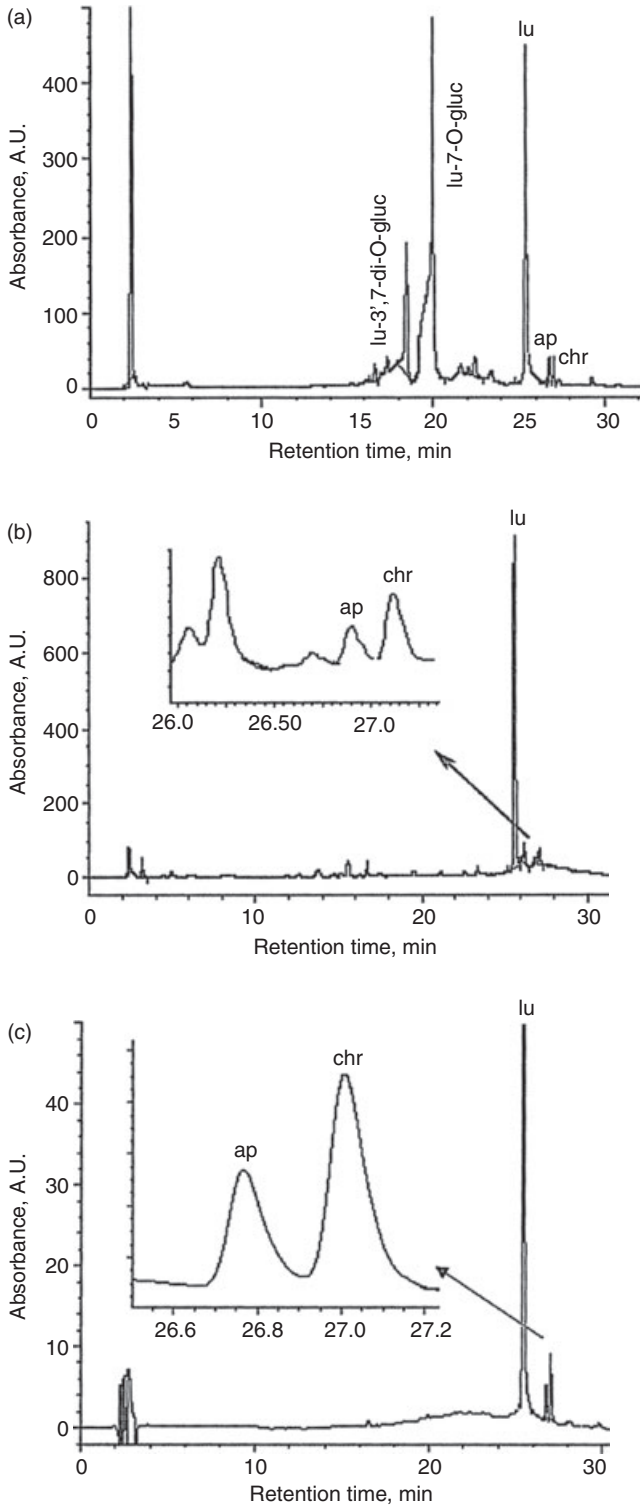

Figure 1 Chromatograms of non-hydrolysed weld extract (a), acid-hydrolysed weld extract (b) and acid-hydrolysed weld-dyed wool extract (c)

the fresh leaves) and as three glucosides. Kaempferol, quercetin and galangin are also present. It is also the source of CI Natural Yellow 12. On the one hand, 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 especially 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 fifteenth-century prayer rug, which is now present in the Türk ve İslam Eserleri Müzesi in Istanbul [14]. This dye source was also used in the Turkish carpets from the fifteenth to seventeenth centuries [29].

In 1971, Tanker and Ertan [30] published a paper about the chemistry of the anthraquinone derivatives in the bark of *Rhamnus petiolaris*. A new acylated flavonol, glycoside, from the berries of *R. petiolaris* was isolated in 1994 by Çalış and colleagues [31]: rhamnetin 3-*O*-[3′′′′-*O*–(*p*-coumaroyl)-α-L-rhamnopyranosyl (l → 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 the *R. petiolaris* plant [32].

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 HPLC with diode-array detection to shed some light on 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.

Addtionally, this work – using three yellow dye plants: weld (*Reseda luteola* L.), buckthorn (*Rhamnus petiolaris* Boiss) and bastard hemp (*Datisca cannabina* L.), all of which are natural dye sources – focuses on dyeing protein fibres that can have an important advantage with regard to 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 [33–35].

Experimental

Materials

One hundred per cent 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, 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 (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-metyl anthraquinone), apigenin (5,7,4′-trihydroy-flavone), datiscetin (3,5,7,2′-tetrahydroxyflavone), quercetin-3-arabinosid and luteolin-7-glucoside from Carl Roth (Germany). Alum [$KAl(SO_4)_2.12H_2O$], hydrochloric acid and methyl alcohol were purchased from Merck (Germany).

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 of 40:1. Wool samples were then squeezed and dried at room temperature for 4 days.

Dyeing

Dyeing was performed by using 20% buckthorn (dried berries), 50% bastard hemp (stem) and 50% weld (above-ground parts) dye plants to textile (w/w). First, the dye plants were separately soaked in 200 ml of deionised boiled water. The mordanted wool samples were heated in a dyebath ratio of 100:1 at 100 °C for 30 min. The wool samples were then removed from the dyebaths, 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 and a G1315A diode-array detector. A G1322A vacuum degasser and a G1316A thermostatted column compartment were also used. Chromatograms were obtained by scanning the sample from 191 to 799 nm with a resolution of 2 nm; eluted peaks were monitored at 255 and 350 nm. Data were analysed using an Agilent Chemstation. A Nova-Pak C_{18} analytical column (3.9 × 150 mm, 4 μm particle size; part no. WAT 086344; Waters, Ireland) 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 [36,37]. Chromatographic separations of the hydrolysed samples were performed using a gradient elution programme that utilises two solvents – solvent A: H_2O – 0.1% trifluoroacetic acid (TFA); and solvent B: acetonitrile (CH_3CN) – 0.1% trifluoroacetic acid. The flow rate was 0.5 ml/min and the elution program was as described earlier [36,37].

Extraction procedure for HPLC analysis

The extraction from the dye plants and the dyed wool samples was carried out as described in earlier reports [33–35]. The samples were prepared as follows.

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

Procedure 1: In the first procedure, the dye extraction from the dye plants (2.4–10.2 mg) was achieved in a 400-μl mixture of methanol/water (2:1; v/v) in a conical glass tube without heating (non-acid hydrolysed).

Procedure 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 water/methanol/37% hydrochloric acid (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 a 400-μl mixture of methanol/water (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 into the HPLC apparatus.

Results and Discussion

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 analysed qualitatively by a reverse-phase HPLC with diode-array detection. The dye extractions for HPLC analysis were carried out using published procedures [36,37]. 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 diode-array detection (monitored at 255 and 350 nm) (Figure 1b,c). The main ingredients of the yellow dye source weld (*R. luteola* L.) are well documented; luteolin [25,38] 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 [38] and chrysoeriol [39] (Figure 1a). 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], it could be detected here.

Buckthorn (*Rhamnus petiolaris* Boiss)

In the sample derived from the non-hydrolysed *R. petiolaris* Boiss extract, the first eluted peak, shown as qu-3-arab in Figure 2a, was identified by comparison with pure quercetin-3-arabinosid compound. The other three peaks were: quercetin glucosides: qu-gluc-1 (λ_{max}: 256, 349), qu-gluc-2 (λ_{max}: 257, 267, 295, 356) and qu-gluc-3 (λ_{max}: 255, 267, 297, 355). The HPLC diode-array detection chromatogram (monitored at 255 nm) obtained from the acid-hydroysed buckthorn extract is shown in Figure 2b. 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 [32]. The main colouring components related to the hydrolysis plant extract were identified as isorhamnetin and rhamnetin, with a minor amount of emodin also present.

Figure 2c shows the HPLC chromatogram collected for the sample extracted from the buckthorn-dyed wool

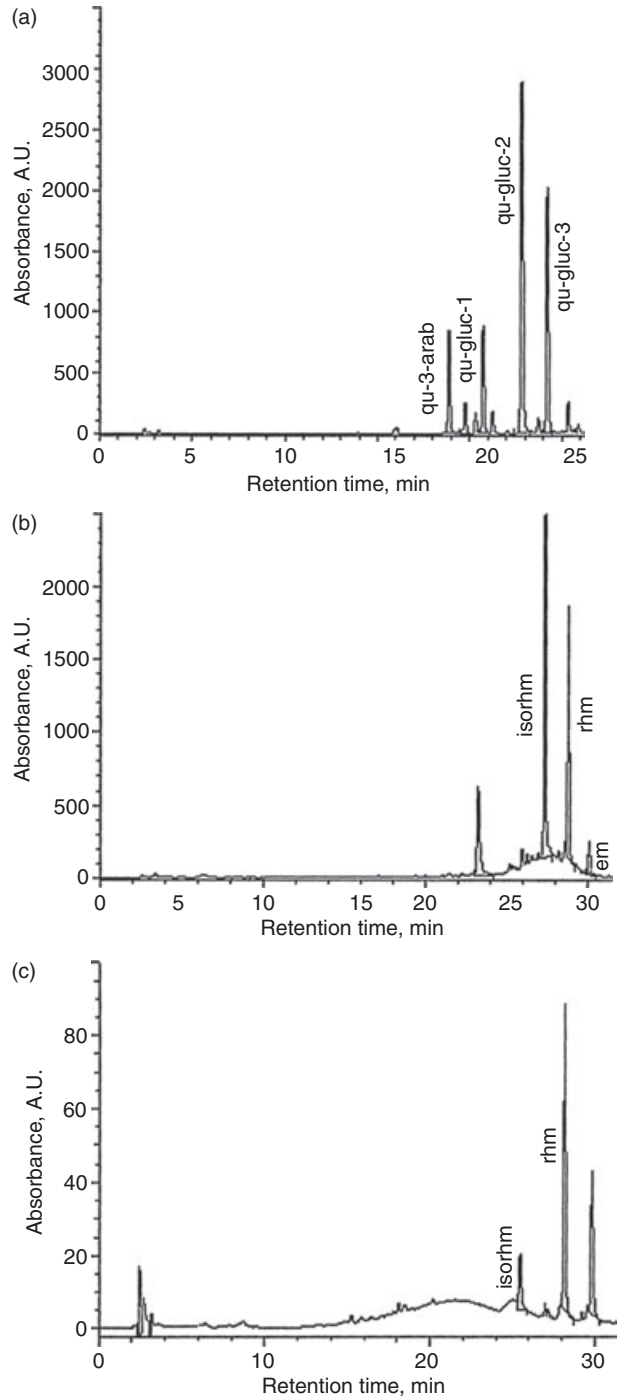

Figure 2 Chromatograms of non-hydrolysed buckthorn extract (a), acid-hydrolysed buckthorn extract (b) and acid-hydrolysed buckthorn-dyed wool extract (c)

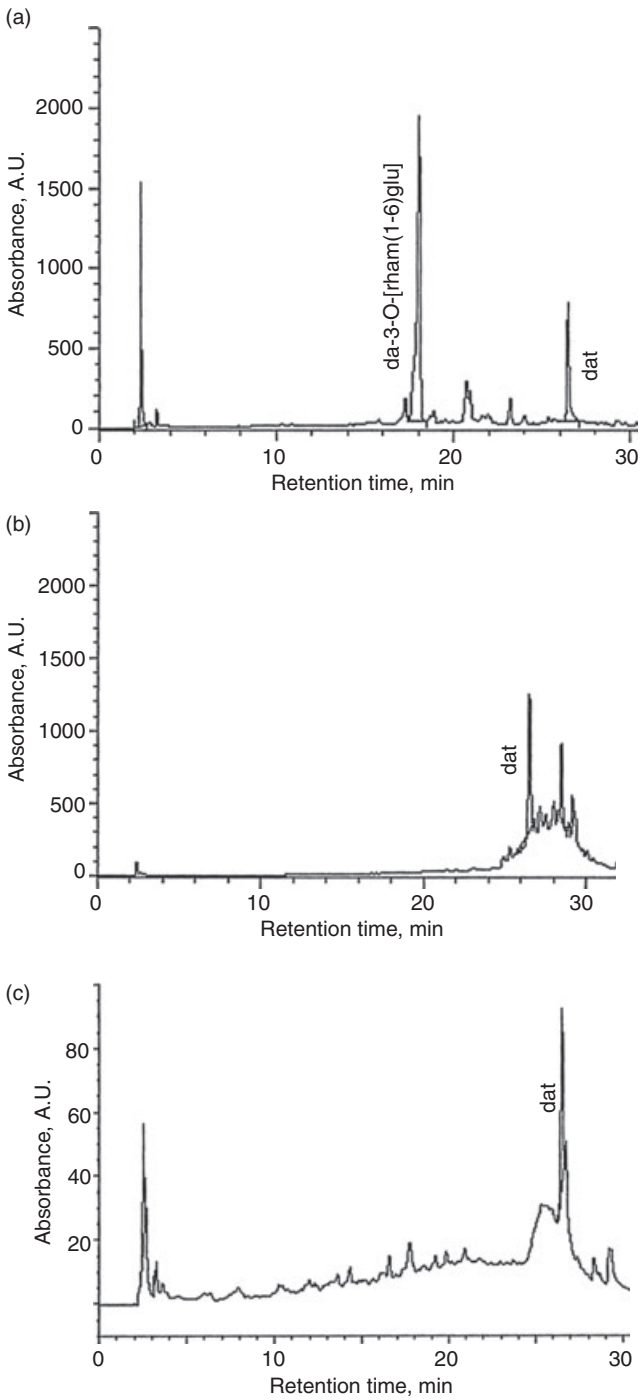

Figure 3 Chromatograms of non-hydrolysed bastard hemp extract (a), acid-hydrolysed bastard hemp extract (b) and acid-hydrolysed bastard hemp-dyed wool extract (c)

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 colouring component.

Bastard hemp (*Datisca cannabina* L.)

The HPLC diode-array detection chromatograms related to the acid-hydrolysed *D. cannabina* L. extract and the dyed-wool extract are shown in Figure 3b,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 the datiscetin compound, which is detected in these extracts, the main peak was detected in the HPLC diode-array detection analysis of sample prepared (Figure 3a) with a solution of methanol/water (2:1) mixture. In their book, Campos and Markham [40] mentioned that the spectrum investigation of various existing natural compounds seems to be in harmony with the spectrum of the main peak detected component (λ_{max}: 257, 305 and 329) datiscetin-3-*O*-[rhamnosyl(1-6)glucoside].

Table 1 Chromatographic and spectral characteristics of the investigated sample extracts

Dye (standard)	Abbreviation	Retention time in the given protocol (min)	UV–vis data
Datiscetin-3-*O*-[rhamnosyl (1-6)glucoside]	da-3-*O*-[rham(1-6)gluc]	18.0	259, 305, 331
Luteolin-3′,7-di-*O*-glucoside	lu-3′,7-di-*O*-gluc	18.4	240, 268, 341
Quercetin-3-arabinosid	qu-3-arab	19.4	255, 267, 295 355
Luteolin-7-*O*-glucoside	lu-7-*O*-gluc	19.9	254, 267, 349
Luteolin	lu	25.5	253, 267, 289, 347
Datiscetin	da	26.4	257, 305, 347
Apigenin	ap	26.5	265, 293, 337
Isorhamnetin	isorhm	26.9	255, 301, 369
Chrysoeriol	chr	27.1	250, 268, 289, 346
Rhamnetin	rhm	28.1	255, 299, 371
Emodin	em	31.7	253, 267, 289, 439

Table 2 Identified dyes in the sample extracts

	Dyes detected		
	Plant		Natural Dyed Silk Samples
Sample	Non-hydrolysed extract	Acid-hydrolysed extract	Wool (acid-hydrolysed extract)
Weld	Luteolin-3′,7-di-*O*-glucoside Luteolin-7-*O*-glucoside Luteolin Apigenin Chrysoeriol	Luteolin Apigenin Chrysoeriol	Luteolin Apigenin Chrysoeriol
Buckthorn	Quercetin-3-arabinosid Possible quercetin glucosides	Isorhamnetin Rhamnetin Emodin	Isorhamnetin Rhamnetin
Hemp	Datiscetin Datiscetin-3-*O*-[rhamnosyl (1-6)glucoside]	Datiscetin	Datiscetin

Conclusions

Chromatographic and spectral characteristics of the investigated sample extracts are displayed in Table 1. 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. Identified dyes in the sample extracts are given in Table 2. The outcome of this study is that it would be possible to analyse and to identify the natural dyes present in historical textiles using a simple method.

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