ORIGINAL

From Plant Extracts to Historical Textiles: Characterization of Dyestuffs by GC–MS

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Abstract A gas chromatographic–mass spectrometric procedure for the analysis of dyes from plant extracts was optimized and applied for the detection of components in extracts from 12 dyeing plants native from all over the world. The main marker molecules in each of the dyestuffs, as well as accessory compounds, were successfully separated and identified by means of their electron impact mass spectra, thus demonstrating that a single GC–MS procedure can be conveniently applied to the detection of natural dyes such as flavonoids, neoflavonoids, anthraquinones and tannins. Other constituents of plant extracts, such as organic acids, oils and sugars, that hydrolyse during the extraction process, were also detected and recognized in the same chromatographic run. The GC–MS method was tested on woollen references dyed according to traditional recipes, and on historical wool samples taken from a tapestry of the sixteenth century, and the main dyestuffs used for colouring could be traced even with the low amounts usually available with the archaeometric samples. Besides some limitations, that are addressed, the results show that the GC analysis is a useful tool for quick assessment and control of natural extracts and the application of the technique for the characterization of dyes in historical textiles, in addition or in place of the more widely used procedures that employ liquid chromatography, is encouraged.

Keywords Gas chromatography · Mass spectrometry detection · Natural dyes · Plant extracts · Flavonoids · Anthraquinones · Tannins · Archaeometric applications

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Introduction

Dyes extracted from locally available sources such as plants, insects and shellfish have been used in every part of the world since ancient times. They have been employed to colour textiles and other objects by fixing them on a variety of substrates [1, 2]. The use of natural dyes suffered a rapid decline that began with the serendipitous preparation of the first aniline dye by Perkin in 1856 as few years later, the inexpensive, charming and stable colours from the chemical industries flooded the market and the use of natural dyes was abandoned in Europe [3]. Since the last decade a greater attention on safety and environmental aspects of chemical industry lead an increasing number of consumers towards "green" products. The relevance of natural resources including natural dyes is therefore rapidly increasing within the modern market, at least for application in specific fields.

Thus, wider availability of products for dyeing purposes obtained from plants through large-scale extraction processes is presently observed [4]. These are ready-to-use powders which contain the same chemical components that could have been present in an ancient dye-bath. Besides other applications, commercially available extracts from dyeing plants have been recently employed in the restoration of historical textiles. An important example are the coloured yarns inserted into the lacunae of one tapestry belonging to the well-known series named "*The Lady and the Unicorn*", exhibited at the *Musée National du Moyen Âge* in Paris. The most relevant requirement for dyeing materials employed for the restoration of objects from the cultural heritage is the possibility of obtaining successfully, with ease and rapidity, a large set of target colours. In addition, they must have no adverse impact on human health. Commercial extracts from dyeing plants can meet

such requirements, provided that a careful selection of raw materials and preparation technologies is performed. Therefore, an important step in the production cycle is the accurate recognition of the chemical species that contribute to impart the final colour to the textile to be dyed. Plant extracts can also be employed as reference materials for the identification of natural dyes in historical textiles, in particular when new analytical procedures are developed.

The analysis of natural dyes in various matrices is generally performed by high-performance liquid chromatography (HPLC) coupled with photo-diode array detector (DAD) and mass spectrometry (MS) with atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI) interface [5, 6]. However, due to the wide range of marker molecules and their different relative amounts that can be found in extracts from natural dyes, their analysis can take advantage of the unique separation performances of gas chromatographic capillary columns and of the high reproducibility of mass patterns achieved by means of electron impact ionization, when the GC is coupled with mass spectrometric detection. An exhaustive literature review [7] of the chromatographic methods for the analysis of flavonoid species, including free aglycones, in various matrices has shown the primary influence of the preliminary preparation steps (extraction, derivatization) on the reliability of the results which may be obtained, highlighting that optimum conditions are dependent on the matrix and the compounds herein present. GC–MS proved to give satisfactory results in all cases examined with excellent resolution and, subsequent to derivatization, sensitivity. In the case of isoflavones a practical GC–MS method was proposed for their determination at low levels in human urine, involving a preliminary cleanup procedure and derivatization with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) [8].

GC–MS has been also proposed for analysis of naturally occurring anthraquinone-type compounds in their silylated forms [9]. Anthraquinone molecules are present in many *Rubiaceae* plants, and particularly in madder roots which have been used since ancient times for dying textiles. In a study about the identification and quantification of the constituents of madder root the results from gas chromatography and high-performance liquid chromatography were compared with the conclusion that it was practically impossible to declare the unambiguous advantage of either one [10]. The HPLC methods certainly prevail for quantification of previously identified constituents of the plant, but in such case separate elutions on different columns and different detections must be applied in order to achieve acceptable results. On the other hand, GC–MS appears to be the preferred method when the plant constituents are of unknown origin since this method allows identification and quantification in a single run, simultaneously [10].

The need to hydrolyze the glycosylated components of the plant extracts and to derivatize the dye molecules for overcoming their low volatility has limited, until now, the development of GC methods for application in the study of historical textiles. The lack of extensive spectral libraries containing marker molecules characteristics of natural dyes is another fact that has inhibited wider use of electron impact mass spectrometry in the archaeometric study of textile materials. This is a serious oversight because, if we consider a complex matrix such as an historic fabric, the possibility to operate with a single method for identification of multiple classes of dyes can represent a great advantage. Furthermore, the separation power and sensitivity of GC–MS turns out to be of help for the characterization of the decomposition products of natural dyes, as demonstrated in a study of aged and non-aged wool specimens dyed with flavonoids obtained from raw plant materials [11].

In this work, we present the application of a common GC–MS method for the identification of the chromophore molecules from plant extracts covering all the major chemical classes of mordant dyes (flavonoids, neoflavonoids and anthraquinones) and some of the plants used as sources of tannins. Commercial extracts obtained from 12 different dyeing plants were considered, coming from species native from all over the world, and they are summarized in Table 1. Most of these plants have been exploited in the past as dye sources and can be promisingly considered for a number of colouring applications also in present days. Prior to the GC–MS analysis the extracts were subjected to proper derivatization reactions in order to overcome the limited volatility of the investigated species [24, 25]. The conditions for the derivatization step were investigated in order to set up the best conditions for the different analytes and the separation of the main colouring molecules was checked on standard mixtures of pure molecules. After that, the optimized procedures were used for the analysis of plant extracts in order to evaluate the possible interferences of the complex analytical matrix in the separation and detection of the dyes. The presence of partially derivatized species has been properly considered and the identification of the molecules which determine the dyeing properties of the extract was achieved by means of the electron impact mass spectra. The GC method has been then tested in the analysis of reference wool samples dyed with old fustic, Persian berries, dyer's broom and madder. These reference wool samples allowed to verify if the GC conditions employed for the plant extracts are applicable to textile matrices. Finally, for assessing the procedure in archaeometric applications, measurements were performed on historical samples from a Brussel tapestry of the sixteenth century.

The strong and weak points of the GC–MS technique for analysis of some of the most popular dyes used in antiquity

Table 1 Plant species, colorant classes, and codes of plant extracts investigated

Plant	Chemical class	Plant code	Habitat and phytochemicals information	References
Genista tinctoria L.	Flavonoids	GT	Plant from the *Fabaceae* family, containing genistein, genistin, puerarin, daidzin and sissotrin as main isoflavones. Luteolin and apigenin are present as minor components	[1]
Reseda luteola L.	Flavonoids	RL	RL (Weld) is a biennial or polycarpic perennial herbaceous plant native of Euro-Asia and naturalized in the Mediterranean region. Its main dyes are luteolin and apigenin in glycosylated forms	[12]
Solidago canadensis L.	Flavonoids	SC	Perennial herb native of North America and naturalized in Europe; contains quercetin, kaempferol and many glycosides of quercetin such as quercitrin, rutin, quercetin-3-rutinoside	[13]
Rhamnus petiolaris Boiss.	Flavonoids	RP	Native to Asia Minor and cultivated in orchards in Turkey. Its berries contain rhamnetin, quercetin, kaempferol aglycones together with smaller quantities of isorhamnetin, rhamnazin, rhamnocitrin and minor amounts of anthraquinones, mainly emodin	[1]
Haematoxylum campechianum L.	Flavonoids	HC	Member of the *Leguminosae pea* family, commonly called Campeache wood or Logwood; it is a small bushy tree that grows naturally in tropical regions of South and Central America and southern Africa. The dyestuff originates in the yellow heartwood which contains haematoxylin. When exposed to air, the red/violet compound haematin is formed	[1]
Caesalpinia echinata Lam.	Flavonoids	CE	A specie of Brazilian timber tree in the *Fabaceae pea* family. Common name is Brazilwood, term also applied to other species used as sources of the colouring matter brazilin (such as Caesalpinia sappan, native to Southeast Asia)	[1]
Rubia tinctorum L.	Anthraquinones	RT	Native to Middle East and eastern parts of the Mediterranean, naturalized in southern and central Europe. Roots contain up to 30 anthraquinones as secondary metabolites; the main colouring matter is alizarin, present as aglycone and as primeveroside of ruberythric acid, in variable quantities depending on the maturity of the plant. The fresh roots contain also purpurin, pseudopurpurin, rubiadin, munjistin, christofin, quinizarin, as well as glycoside forms such as rubianin, and lucidin glycoside	[10, 14]
Rhamnus frangula L.	Anthraquinones	RF	Bark and leaves yield a yellow dye named Alder Buckthorn. The berries, when unripe give a green colour readily taken by wool; when ripe they give various shades of blue and grey. Frangula bark contains emodin di- and mono-glycosides such as the diglycosides glucofrangulin A (emodin-6-0-α-L-rhamnosyl-8-0-β-D-glucoside) and glucofrangulin B (emodin-6-0-β-D-apiosyl-8-0-β-D-glucoside); the monoglycosides frangulins A, B, C (emodin-6-0-α-L-rhamnoside, emodin-6-0-β-D-apioside, emodin-6-0-β-D-xyloside) and emodin- 8-0-β-D-glucoside, as well as dianthrones (such as chrysophanoldianthrone and chrysophanolphys-ciondianthrone); the latter are particularly abundant in fresh callus	[15–17]
Castanea sativa Mill.	Tannins	CS	Known as sweet chestnut, had great diffusion in Europe, north Africa, Pakistan, India, Nepal, China and Indonesia. Main chemical constituents of heartwood of interest for dyeing purposes are: gallic acid, ellagic acid, catechin, epicatechin, gallocatechin, epigallocatechin, ferulic acid, vanillic acid, aromatic aldehydes (vanillin, syringaldehyde, coniferaldehyde and sinapaldehyde), coumarins (esculetin, umbelliferone, scopoletin and methylumbelliferone scopoletin) and glycoside of quercetin and kaempferol	[18]
Acacia catechu L.	Tannins	AC	Member of the *Febaceae* family, diffused throughout Asia. It contains: catechin, epicatechin, epicatechin gallate and procatechinic acid. Glycosides of quercetin, kaempferol and afzelechin are also present as minor compounds. Catechin in this plant plays a vital role as antioxidant	[19]

Table 1 continued

Plant	Chemical class	Plant code	Habitat and phytochemicals information	References
Gallnuts from *Rhus semialata* Murr.	Tannins	RS	Also called *Rhus chinensis Miller* or *Rhus javanica L.*, Asiatic tree of the Anacardiaceae family. The vernacular name is Sumac, used to indicate every specie of the Rhus genus. The galls of *Rhus* are induced by aphids; 14 types of gallnuts induced by different species of aphids have been described in the literature. Walls of the galls may contain up to 70 % tannins and give compounds of considerable commercial value, particularly tannic and gallic acid. *Rhus semialata* was mainly used in China and Japan, where it is considered a prominent resource for the production of tannins	[20]
Terminalia chebula Retz.	Tannins	TC	Plant belonging to the *Combretaceae* family. Native in India and Southeast Asia and extensively cultivated in Taiwan. It contains about 30 % of hydrolysable tannin, encompassing 14 components (gallic acid, chebulic acid, punicalagin, chebulanin, corilagin, neochebulinic, ellagic acid, chebulegic acid, chebulinic acid, 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose, 1,6,-di-*O*-galloyl-D-glucose, casuarinin, 3,4,6-tri-*O*-galloyl-D-glucose and terchebulin). Phenolic compounds, flavonol glycosides and triterpenoids, have been also isolated and ethyl gallate and luteolin were detected as well	[21–23]

are considered in this work, in view of the possible use of the analytical procedure for both quality control of the industrial production of natural extracts and for the characterization of dyes in historical textiles.

Experimental

Chemicals and Materials

Purpurin analytical grade was purchased from Fluka Chemical (Buchs, Switzerland); luteolinapigenin, kaempferol, quercetin, genistein, emodin, alizarin, catechin, gallic acid and ellagic acid were obtained from Sigma Aldrich (Milan, Italy). Tridecane, used as internal standard, was purchased from Carlo Erba (Milan, Italy). Ethyl acetate Baker Analyzed™ grade was purchased from J.T. Baker Chemical Co. (Deventer, NL). *N,O*-Bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) + 1 % trimethylchlorosilane (TMCS) reagent was from Supelco (Bellafonte, PA, USA). Plant extracts, reported in Table 1, were provided by Couleurs de Plantes (Rochefort, France).

The reference wool samples were provided by "*Associazione Colore e Tintura Naturale Maria Elda Salice*" (Milan, Italy). The samples are part of a collection of fleece dyed in the last 5 years using fresh natural dyestuffs and alum as mordant and with addition of cream of tartar, according to traditional procedures. The dyestuffs used are: Persian berries (buckthorn family), old fustic (heartwood of dyer's mulberry), dyer's broom (*Genista tinctoria* L.), madder (root of *Rubiaceae* or *Galium* families). More exact botanic information on dye sources and on dyeing procedures are not available.

The historical samples have been obtained from a Brussels tapestry of sixteenth century named "Deer hunting with mythological figures" which decorates a wall of Villa Necchi Campiglio in Milan (Italy), an historical building now owned by FAI (Fondo Ambiente Italiano) [26]. The samples, a brown-reddish yarn (sample CC 11) and a clear yellow yarn (sample CC6), have been taken from the back of the tapestry during the restoration work at the Centro Conservazione e Restauro La Venaria Reale (Turin, Italy).

Derivatization Procedure

The derivatization of the dyes was performed with BSTFA + 1 %TMCS at 70 °C. Standard dye solutions were prepared in ethyl acetate, at 25 µg mL^{-1} concentration. 120 µL of derivatization reagent were added to 200 µL of each standard solution. After the derivatization 1 µL of tridecane in ethyl acetate (1 µg mL^{-1}) was added as internal standard. 2 µL of each final solution were injected into the GC apparatus. Due to the low solubility of ellagic acid in ethyl acetate, 0.13 mg of this compound was first derivatized in 200 µL of BSTFA + 1 % TMCS, and 400 µL of ethyl acetate was subsequently added to 10 µL of the derivatized solution.

The derivatization reaction was monitored at 30, 60, and 75 min by considering a standard mixture of molecules representative for the chemical classes of dyes here considered. The ratio of the peak area of the analytes over the peak area of the internal standard was used for semi-quantitative analysis.

In the solutions obtained from the plant extracts, the quantity of derivatization reagent was increased in order to promote maximum derivatization of analytes. In particular,

120 μL of BSTFA and 1 %TMCS were added to 0,02 mg of each dry extract dissolved in 200 μL of ethyl acetate, and the derivatization reaction at 70 °C was extended to 75 min.

Preparation of References Wool Samples and Historical Samples

The reference wool samples, having a weight of about 15 mg, were treated with 600 μL MeOH and 20 μL HCl (30 %) at 80 °C for 65 min. The solution was purified on Nylon filter and then dried under a soft stream of nitrogen. 100 μL of the derivatization agent BSTFA + 1 % TMCS and 100 μL of ethyl acetate were added. The derivatization reaction was performed at 70 °C for 75 min. 2 μL of final solution was injected in the GC apparatus. The same procedure was applied for extraction of the chromophores from the historical tapestry; the wool samples in this case were much smaller, with weight of about 2 mg each.

Gas chromatographic Apparatus

The GC–MS was a 6,890 N Network GC System (Agilent Technologies USA) mounting a methyl phenyl polysiloxane cross-linked 5 % phenyl methyl silicone (30 m, 0,25 mm internal diameter, 0,25 μm film thickness) capillary column. The mass spectrometer coupled to the GC apparatus was a 5973 Network Mass selective Detector (Agilent Technologies, USA). Mass spectra were recorded under electron impact at 70 eV (scan range 40–700 m/z). The interface was kept at 280 °C, the ion source at 230 °C and the quadrupole mass analyser at 150 °C.

The GC conditions, which were optimized on standard mixtures of pure compounds, were as follows: initial temperature 57 °C, 2 min isothermal, ramp at 10 °C min^{-1} up to 200 °C, 3 min isothermal, ramp at 20 °C min^{-1} up to 300 °C and isothermal for 20 min. Helium (1.2 mL min^{-1}) was employed as carrier gas). The GC–MS system was computer-controlled by the Enhanced Chem Station software (Agilent Technologies, USA).

Results and Discussion

Derivatization

A number of dyes from natural sources are polyphenols (Fig. 1), which are often present in plants as glycosides [27]. They hydrolyze into aglycones and saccharides during the extraction processes from the plant (and/or from textile samples). Both glycosides and aglycones need to be derivatized for GC analysis, due to their high polarity and low volatility.

Silylation with BSTFA + 1 %TMCS reagents is one of the most efficient and widely used processes to convert polyphenols and phenolic acids into volatile trimethylsilyl (TMS) derivatives [24, 25]. Sugars may also be detected in GC–MS as trimethylsilyl derivatives and BSTFA is a reagent normally used for their derivatization [28, 29].

The target molecules investigated in this work exhibited a variable number of hydroxyl functional groups. The performance of the derivatization procedure may depend on a number of factors such as temperature and time of reaction, nature of the derivatization agent and solvent. Also the number and the type of reactive functional groups contained in the molecules and the steric hindrance play a role in affecting the ease of the derivatization. Therefore, some among the dyes considered here, a long reaction time may be needed to achieve the derivatization of all their functional groups.

Figure 2 shows the formation trends of the per-TMS derivates. It is evident that alizarin and purpurin were derivatized within 30 min of reaction, whereas flavonoids and tannins would have needed longer time to achieve complete derivatization; after 60 min of reaction the peaks corresponding to the di-TMS isomers of emodin were still detected.

Chromatographic Separation and Identification of Dyes

The gas chromatographic separation of the derivatized dyes was effective and no peak overlapping occurred for the molecules of interest (see Fig. 3 where the chromatogram of a solution of anthraquinone dyes is reported) even when dye extracts, which contain a large number of compounds in addition to the target dyes, are considered. The results for each of the considered extracts are reported and discussed in the following sections. The EI mass fragmentation spectra collected from the derivatized target molecules are reported in Tables 2, 3 and 4. Other markers, that can be associated with the use of a specific natural dye, were also detected in the plant extracts and are included in the tables. Moreover, other natural compounds contained in plants were recognized by comparison with data from spectral libraries (matching results at probability higher than 98 %).

Flavonoids in Plant Extracts of *Genista tinctoria L., Reseda luteola L., Solidago Canadensis L. and Rhamnus petiolaris Boiss.*

Figure 4 illustrates a chromatogram of plant extracts from *Genista tinctoria* (a) and portions of the chromatograms obtained for *Reseda luteola* (b)*, Rhamnus petiolaris* (c) and *Solidago Canadensis* (d).

The chromatogram of the *Genista tinctoria* extract (Fig. 4a) shows a complex picture. Several carboxylic
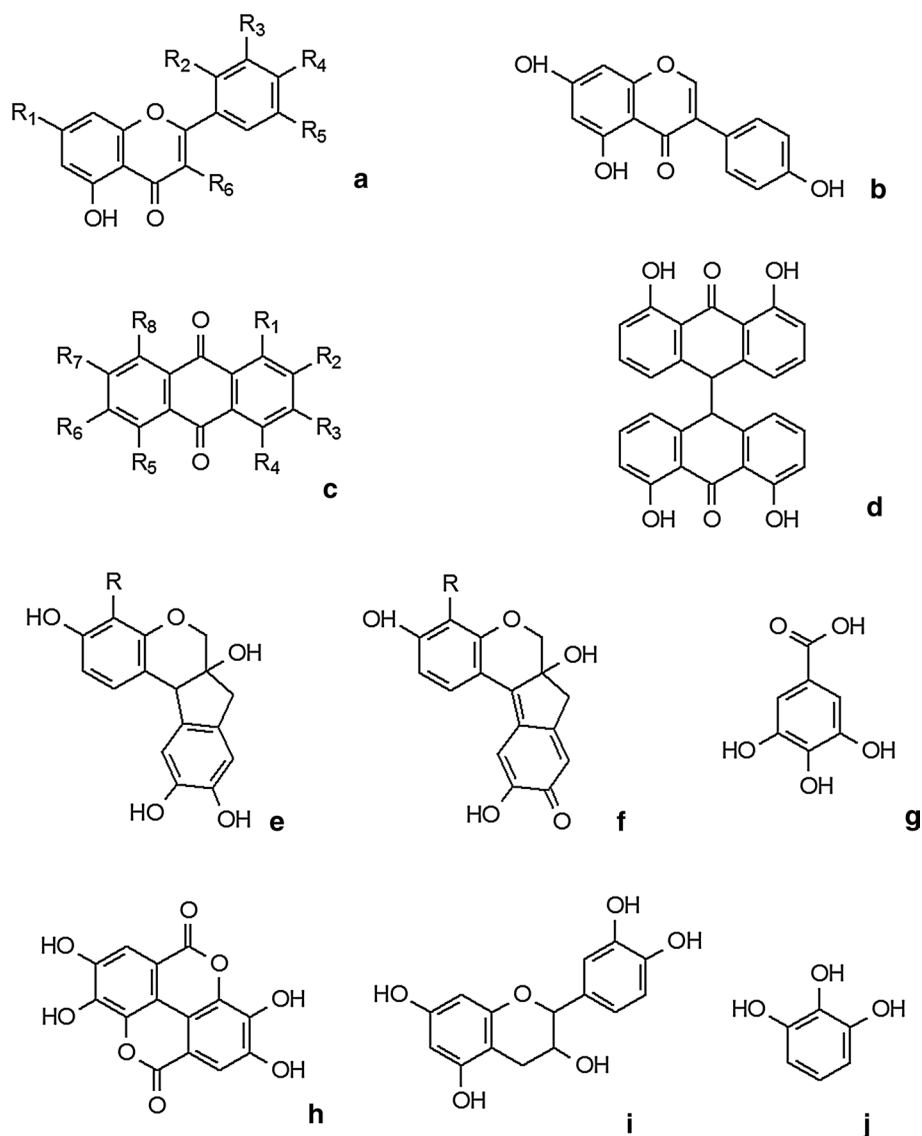
Fig. 1 Structures of flavonoids, anthraquinones and tannins discussed in this paper. **a** Luteolin: R_1 = OH; R_2 = H; R_3 = OH; R_4 = OH; R_5 = H; R_6 = H. Apigenin: R_1 = OH; R_2 = H; R_3 = H; R_4 = OH; R_5 = H; R_6 = H. Ramnethin: R_1 = OCH$_3$; R_2 = H; R_3 = OH; R_4 = OH; R_5 = H; R_6 = OH. Rhamnazin R_1 = OCH$_3$; R_2 = H; R_3 = OCH$_3$; R_4 = OH; R_5 = H; R_6 = OH. Kaempferol: R_1 = OH; R_2 = H; R_3 = H, R_4 = OH; R_5 = H; R_6 = OH. Quercetin: R_1 = OH; R_2 = H; R_3 = H; R_4 = OH; R_5 = OH; R_6 = OH. **b** Genistein: R_1 = OH; R_2 = OH; R_3 = OH. **c** Alizarin: R_1 = OH; R_2 = OH; R_3 = H; R_4 = H; R_5 = H; R_6 = H; R_7 = H; R_8 = H. Pur-

purin: R_1 = OH; R_2 = OH; R_3 = H, R_4 = OH; R_5 = H; R_6 = H; R_7 = H; R_8 = H. Pseudopurpurin: R_1 = OH; R_2 = OH; R_3 = COOH; R_4 = OH; R_5 = H; R_6 = H; R_7 = H; R_8 = H. Lucidin: R_1 = OH; R_2 = COH; R_3 = OH; R_4 = H; R_5 = H; R_6 = H; R_7 = H; R_8 = H. Emodin: R_1 = OH; R_2 = H; R_3 = OH, R_4 = H; R_5 = H; R_6 = CH$_3$; R_7 = H; R_8 = OH. Physcion: R_1 = OH; R_2 = H; R_3 = OCH$_3$, R_4 = H; R_5 = H; R_6 = CH$_3$; R_7 = H; R_8 = OH. **d** Chrysazindianthrone. **e** Brazilin: R = H. Haematoxylin: R = OH. **f** Brazilein: R = H. Hematein: R = OH. **g** Gallic acid. **h** Ellagic acid. **i** Catechin. **l** Pyrogallol

acids were found: group 1 includes the TMS esters of tartaric, malic, malonic, aspartic, threonic, citramalic and succinic acids. A large part of the chromatogram from the *Genista tinctoria* extract is dominated by sugars: monoglycosides (group 2) detected at retention times between 16 and 22 min; oligoglycosides (group 4) eluted at higher retention time according to their molecular weight. The isoflavones detected in the chromatogram are

genistein (peak 6) and a very low peak of luteolin (peak 7), both in the per-TMS form. The derivatization reaction of genistein appears to be incomplete as genistein di-TMS is also present (peak 5). In the extract of *Reseda luteola*, luteolin and apigenin aglycones were recognized (Fig. 4b). Incompletely derivatized forms of luteolin and apigenin are also present (luteolin tri-TMS and apigenin di-TMS).
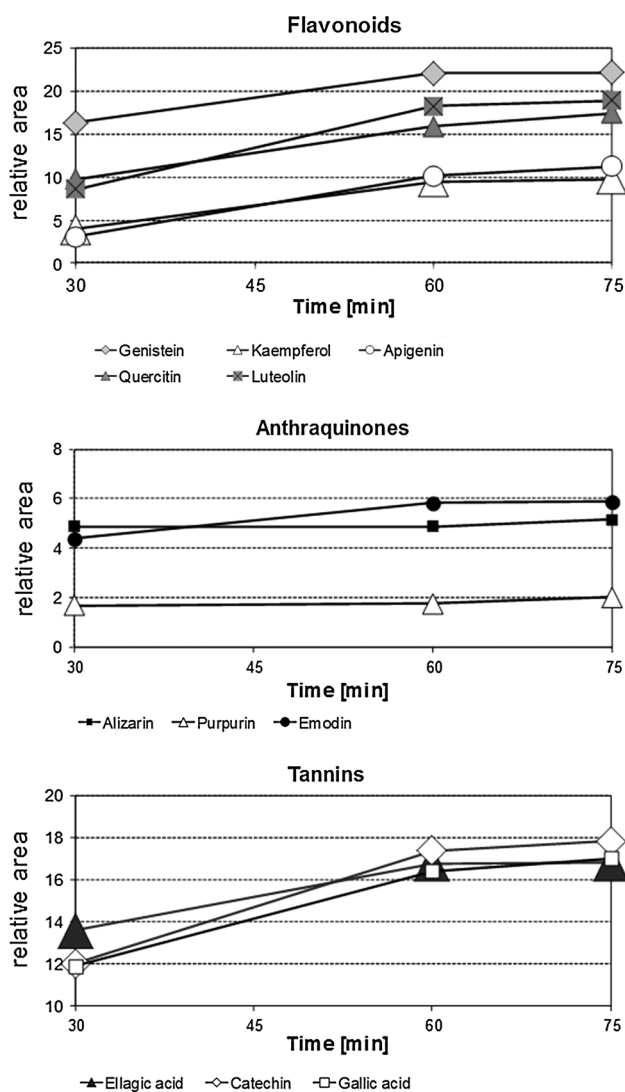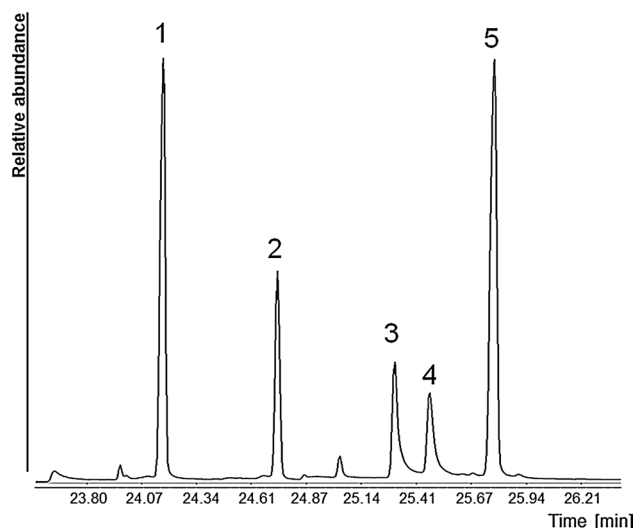
Fig. 2 Relative area of the per-TMS derivative peak in chromatograms of standard molecules in the time range 30–75 min

Fig. 3 Chromatogram of standard solution of anthraquinones. *1* Alizarin di-TMS; *2* purpurin tri-TMS; *3*, *4* isomers of emodin di-TMS; *5* emodin tri-TMS

structures (Fig. 1) with haematoxylin having an extra OH group in the A ring [30, 31].

Neoflavonoids readily oxidize in air and are present in dyeing materials as redox couples, such as haematoxylin/hematein and brazilin/brazilein. *Caesalpinia echinata* and *Haematoxylum campechianum* have been used for centuries as dyestuffs and for preparing lakes and inks [6].

In the chromatogram of the *Haematoxylum campechianum* extract (Fig. 5a) peaks pertaining to different silylated haematoxylins are seen, together with hydroxyhaematoxylin per-TMS.

The chromatogram of the extract from *Caesalpinia echinata* (Fig. 5b) shows the presence of brazilin with two different silylation degrees and a very small peak due to haematoxylin per-TMS.

Anthraquinones in Plant Extracts of *Rubia tinctorum L.* and *Rhamnus frangula L.*

Figure 6a shows a portion of the chromatogram obtained from the *Rubia tinctorum* plant extract, where purpurin and alizarin were identified by the matching of retention times and mass spectra of standard molecules. Although pseudopurpurin is one of the major dyes in the roots of this plant, it was not detected in the extract, possibly because the extraction process has caused the decarboxylation of the molecule converting it into purpurin.

Trimethylslyl sugar derivatives are abundant in the chromatogram of *Rubia tinctorum* extract and their assignment was possible through data reported in literature [28, 29].

Anthraquinones in *Rhamnus* species are formed via polyketide pathway and their structures are characterized

In the *Ramnus petiolaris* extract sample (Fig. 4c) kaempferol, quercetin and rhamnetin were detected, all in their per-TMS form. Rhamnetin partially co-elutes with rhamnazin, although the separation is appropriate for qualitative purposes.

Figure 4d shows portion of the chromatogram obtained from the *Solidago canadensis* extract, where quercetin per-TMS, kaempferol per-TMS, kaempferol tri-TMS, and rhamnetin per-TMS peaks are evident.

Neoflavonoids in Plant Extracts of *Haematoxylum campechianum* and *Caesalpinia chinata*

Brazilin and haematoxylin are neoflavonoids that are found in the hard-woods of *Caesalpinia echinata* and *Haematoxylum campechianum*, respectively. They show very similar

Table 2 Characteristic ionic fragments detected for persilylated flavonoids from analysis of plants extracts

Compound	MW[a]	m/z	Plant [b]
Apigenin	486	471 (100), 399 (5), 228 (10), 73 (25)	RL
Brazilin	574	574 (100), 484 (89), 472 (33), 445 (23), 395 (22), 267 (15), 73 (47)	CE
Genistein	486	414 (100), 339 (60), 192 (14), 73 (14)	GT
Haematoxylin	662	662 (100), 557 (92), 393 (16), 265 (15), 73 (95)	HC
Hydroxyhaematoxylin	678	678 (60), 562 (32), 534 (22), 519 (19), 147 (25), 73 (100)	HC
Kaempferol	574	559 (100), 503 (20), 272 (6), 73 (20)	RP, SC
Luteolin	574	559 (100), 471 (15), 399 (10), 73 (13)	RL
Quercetin	662	647 (100), 576 (20), 559 (14), 73 (46)	RP, SC
Rhamnetin	604	589 (100), 517 (10), 501 (20), 73 (20)	RP, SC
Rhamnazin	546	531 (100), 501 (30), 457 (10), 258 (6), 73 (15)	RP

In brackets the intensity of the ions

[a] Per-trimethylsilylated forms

[b] Codes as in Table 1

Table 3 Characteristic ionic fragments detected for persilylated anthraquinones from analysis of plants extracts

Compound	MW[a]	m/z	Plant[b]
Alizarin	384	371 (100), 339 (10), 296 (9), 73 (25)	RT
Chrysazindianthrone[c]	594	397 (100), 369 (17), 297 (6), 179 (11), 73 (29)	RF
Emodin	486	471 (100), 73 (15)	RF
Physcion	414	413 (100), 296 (5), 199 (5), 73 (16)	RF
Purpurin	472	457 (41), 443 (100), 412 (37), 370 (28), 341 (32), 311 (10), 73 (32)	RT

In brackets the intensity of the ions

[a] Per-trimethylsilylated forms

[b] Codes as in Table 1

[c] Chrysazindianthrone is detected only in di-trimethylsilylated form

Table 4 Characteristic ionic fragments detected for persilylated tannins from analysis of plants extracts

Compound	MW[a]	m/z	Plant[b]
Catechin	650	368 (100), 355 (28), 267 (6), 179 (6), 73 (28)	AC
Gallic acid	458	458 (100), 443 (33), 399 (6), 355 (8), 281 (70), 73 (50)	CS, RS, TC
Ellagic acid	590	590 (100), 575 (82), 487 (10), 73 (95)	TC
Pyrogallol	342	342 (60), 239 (100), 342 (20), 211 (9), 73 (56)	CS

In brackets the intensity of the ions

[a] Per-trimethylsilylated forms

[b] Codes as in Table 1

by substituent groups in both ring A and C. From the bark and leaves of *Rhamnus frangula* a yellow dye named Alder Buckthorn is obtained. The aglycones detected in the buckthorn extract are per-TMS derivatives of physcion and emodin. The literature does not mention physcion among the anthraquinones that are normally found in buckthorn, nevertheless it was detected in the extract here considered (Fig. 6b, peak 10) along with emodin (Fig. 6b, peak 11). Peak 9 in Fig. 6b can be assigned to di-TMS derivative of chrysazindianthrone, which was recognized by considering its mass spectrum (ions are: 594, 576, 561, 397, 369, 73). Also this compound is not mentioned in the literature among the constituents of Frangula. With the available data we are not able to indicate whether chrisazindianthrone was actually present in the plant or it was formed during the extraction process. Moreover, the possibility that the detected peak derives from chrysophanoldianthrone, which loses a methyl group in the ionic source, cannot be excluded.
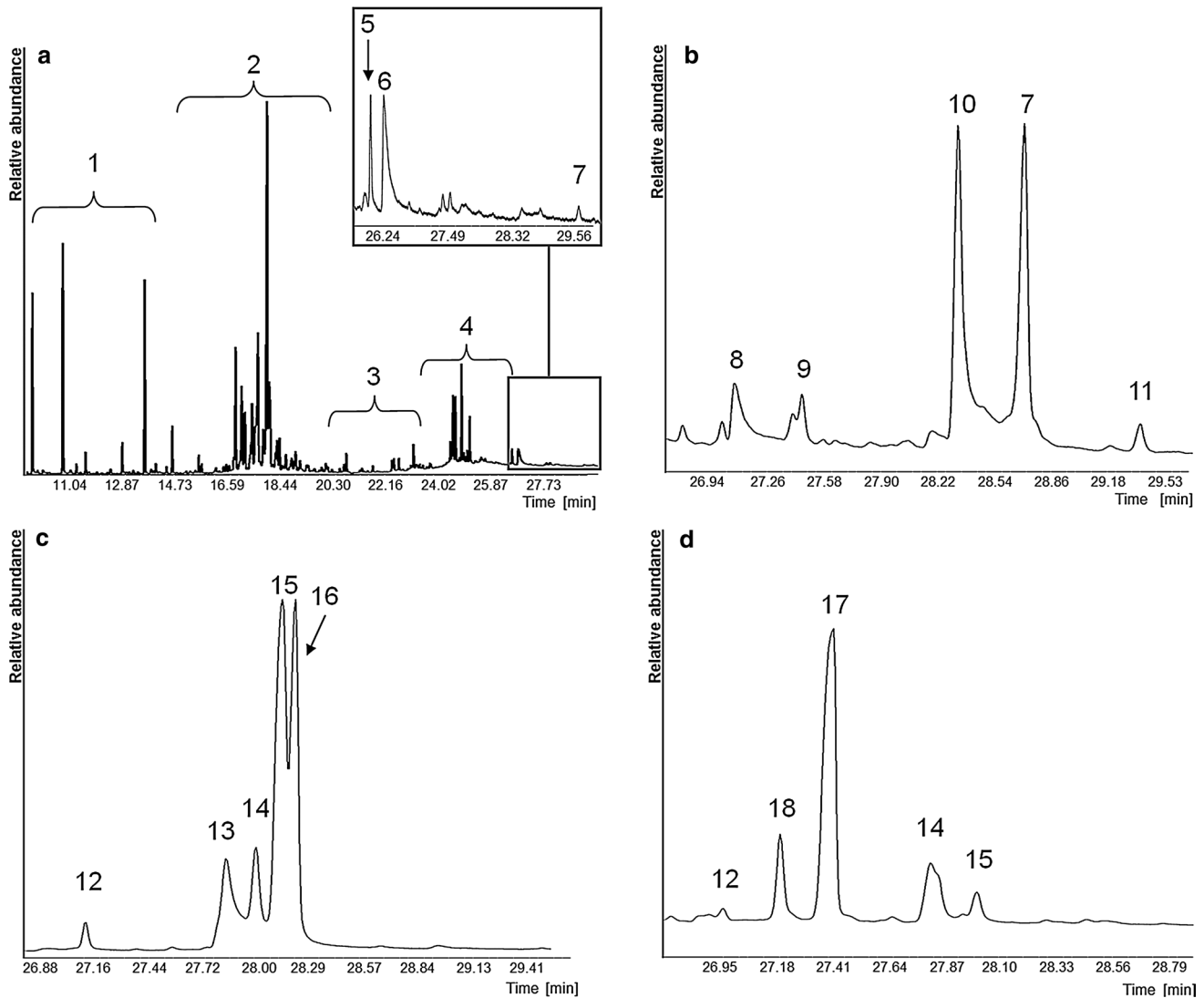
Fig. 4 Chromatograms of plant extracts *Genista tinctoria* (**a**), *Reseda luteola* (**b**)*, Rhamnus petiolaris* (**c**) and *Solidago canadensis* (**d**). *1* Organics acid in TMS form; *2* TMS derivative of mono-glycosides; *3* fatty acids; *4* TMS derivative of oligo-glycoside; *5* genistein di-TMS; *6* genistein per-TMS; *7* luteolin per-TMS; *8* apigenin di-TMS; *9* api-genin per-TMS; *10* luteolin tri-TMS; *11* sitosterol tri-TMS ether; *12* kaempferol per-TMS; *13* rhamnetin tri-TMS; *14* quercetin per-TMS; *15* rhamnetin per-TMS; *16* rhamnazin per-TMS; *17*, *18* unknown compounds

Tannins in Plant Extracts of Castanea sativa Mill., Acacia catechu L., gallnuts From Rhus semialata Murr. and Terminalia chebula Retz.

Terrestrial plant tannins are classified as condensed tannins (polymers of flavan-3-ols) or hydrolysable tannins, also known as proanthocyanidins, as reported elsewhere [32–36].

Condensed tannins are oligomers or polymers of two or more flavanol units, usually catechin and epicatechin (and also prothocyanidin, prodelphin, profisetin, prorobinetinidin). Moreover, other phenolic acids, such as gallic or *p*-coumaric, may be linked to condensed tannins. The extraction processes of tannins from plants normally promote the partial depolymerization of condensed tannins, although fractions of higher molecular weight remain insoluble. Furthermore, acid hydrolysis liberates ellagic acid [36, 37].

The extracts from tannin-bearing plants were selected here taking into consideration both their different geographical origin and their importance as dyes for textiles.

Because of the great diffusion of tannins in plants of various species (see Table 1), it can be difficult to trace their source in textiles. However, tannins can be recognized through GC, as shown in Fig. 7, which reports chromatograms of the extracts from tannin-bearing plants here considered.
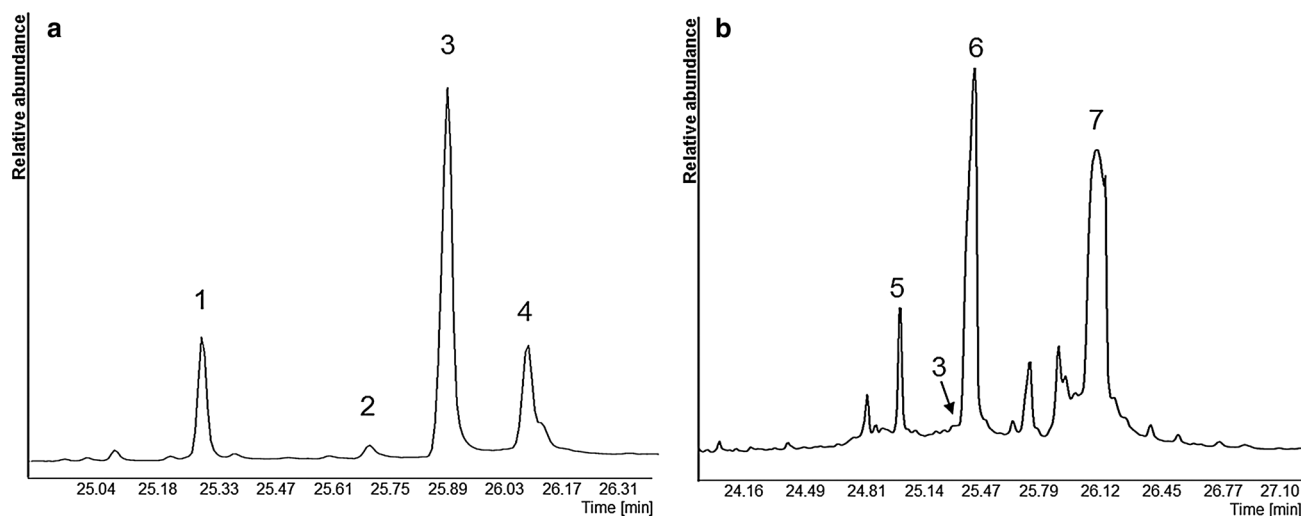
Fig. 5 Chromatograms of *Haematoxylum campechianum* (**a**) and *Caesalpinia echinata* (**b**). *1* Haematoxylin per-TMS; *2* hydroxyhaematein per-TMS; *3* haematoxylin tetra-TMS; *4*, *7* unknown compounds; *5* brazilin per-TMS; *6* brazilin tri-TMS
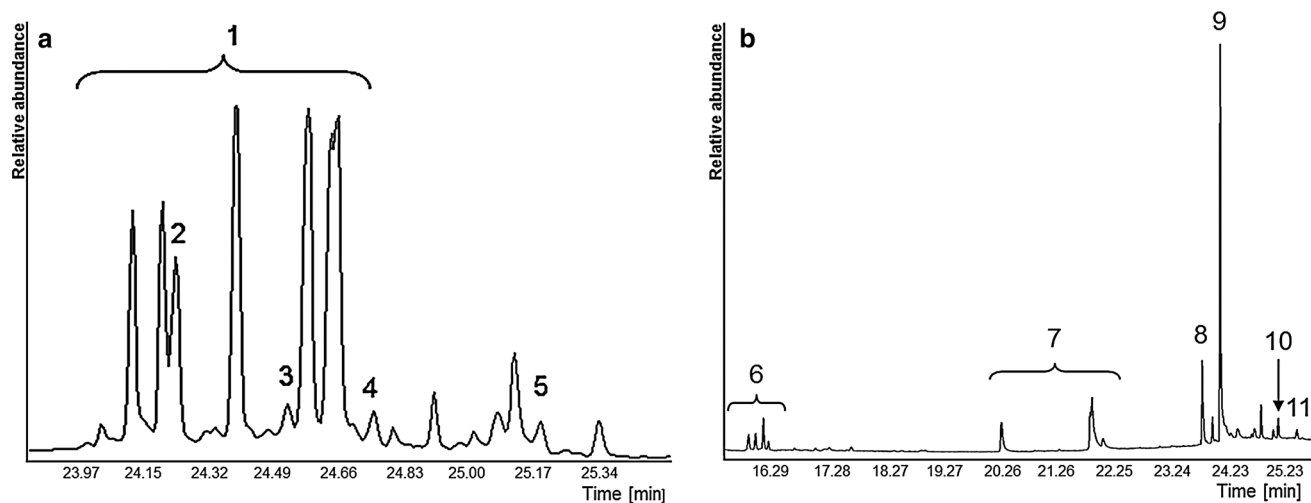
Fig. 6 Chromatograms of *Rubia tinctorum* L. (**a**) and *Rhamnus frangula* L. (**b**). *1* Mono glycosyl in TMS form, *2* alizarin, per-TMS, *3* unknown anthraquinone, *4* purpurin per-TMS, *5* lucidina per-TMS, *6*

polyols, *7* TMS esters of fatty acid, *8* di-*n*-octylpthalate, *9* chrisazin-dianthrone di-TMS, *10* physchion per-TMS, *11* emodin per-TMS

In the *Acacia catechu* extract, only catechin, epicatechin and 1,2 benzenedicarboxylic acid were detected, and no peaks from sugar or fatty acid were present.

Gallic acid is the major component found in the *Castanea sativa* sample, in the extract of gallnuts from *Rhus semialata* and in the *Terminalia chebula* extract. In the *Terminalia* extract, ellagic acid was also found, while in *Castanea sativa*, pyrogalloyl was detected.

Chromatograms of the three samples show that the extracts differ especially in the amount of sugars, which are abundant in *Rhus semialata*, few in the chromatogram of *Castanea sativa* and practically undetectable in

Terminalia chebula, where only a very low peak of glucose was present.

The chromatogram of *Rhus semialata* sample shows two evident peaks (namely, 8 and 9) that correspond to isomers of an unknown compound which was also detected in other gallnut samples previously analysed in our laboratory. A further isomer of the same compound was also detected in *Terminalia chebula* (peak 10). The mass spectra of these unknown compounds contain ions (*m/z* 369, 355, 281, 179, 73) also presents in the fragmentation of gallic acid, which may indicate that they derive from hydrolysis reactions of tannic acid.
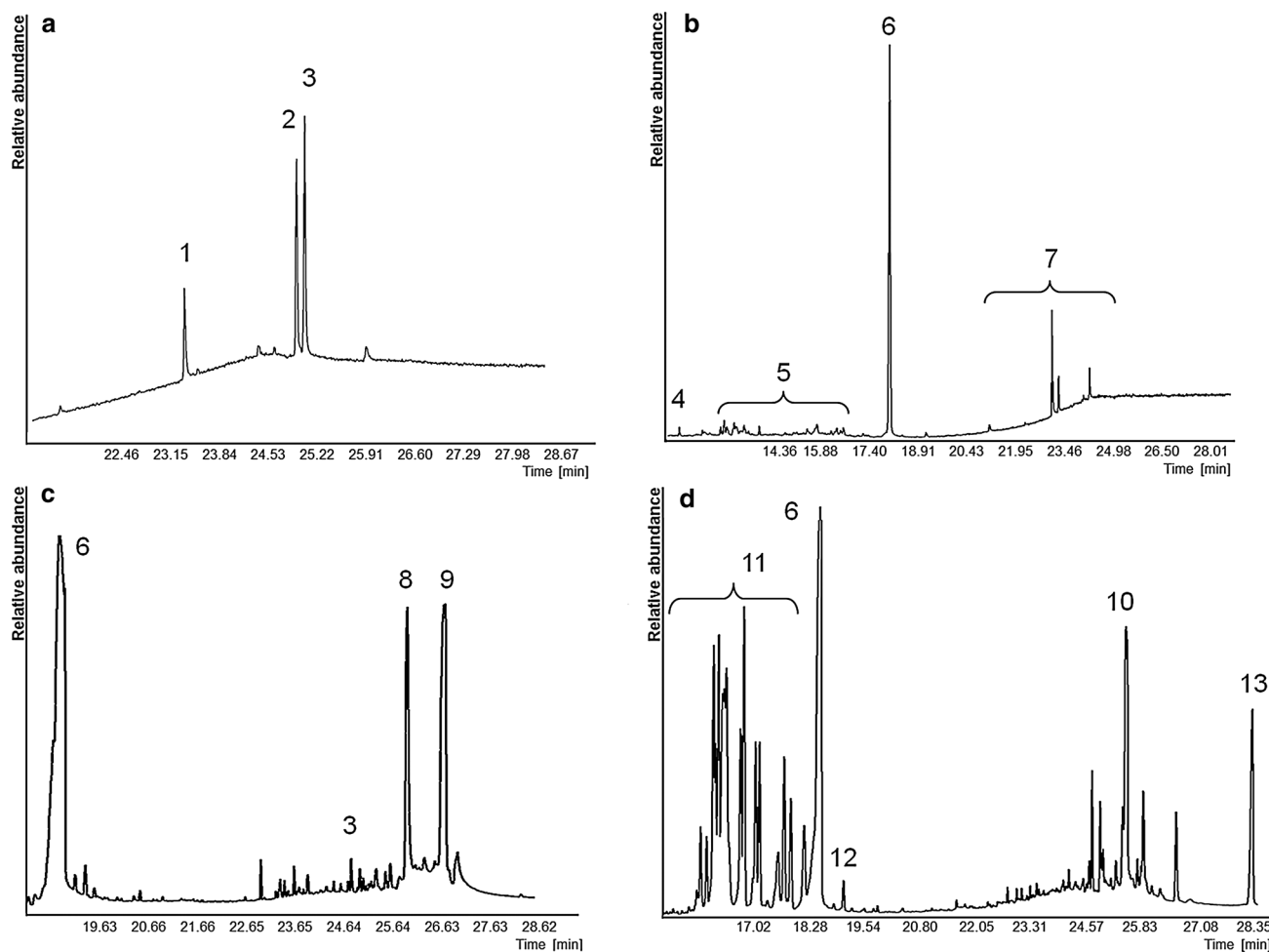
Fig. 7 Chromatograms of extracts: *Acacia catechu* (**a**), *Castanea sativa* (**b**), gallnuts from *Rhus semialata* (**c**) and *Terminalia chebula* (**d**). *1* 1,2-Benzenedicarboxylic acid mono (2-ethylexyl) ester; *2* epi-catechin per-TMS; *3* catechin per-TMS; *4* pyrogallol per-TMS, *5* TMS derivatives of mono-glycosides; *6* gallic acid per-TMS; *7* TMS derivatives of fatty acids; *8, 9, 10* isomers of unknown compound (possibly derivatives from hydrolysis of tannic acid); *11* organic acids in TMS form, *12* glucose TMS, *13* ellagic acid per-TMS

Reference Dyed Wool and Historical Samples

The analytical procedure based on GC–MS was applied to the analysis of references wool samples of about 15 mg of weight and dyed with Persian berries, old fustic, dyer's broom and madder, respectively. Peak assignment was based on comparison with reference compounds and materials, and with use of the NIST 0.8 library mass spectra are based on mass spectra reported in the literature. Analytical results, shown in Fig. 8, revealed the possibility of detecting flavonoids and anthraquinones in the wool samples dyed with the more common dyes. The compounds extracted from wool consist of sugars, fatty acids and sterols whose retention times are distinct from the marker molecules, characteristic of each dye.

In the samples dyed with Persian berries, quercetin and rhamnetin (peaks 6, 7 and 8) were detected as the main chromophores, and emodin (peak 5) was also recognized.

In the chromatogram of samples dyed with old fustic, morin (peak 2) was found as principal colouring component, as well as maclurin (peak 1) whose mass spectrum is shown in Fig. 8a. Kaempferol, a minor component of this dye, could be also recognized (peak 3).

As expected, genistein (peak 9) has been detected as principal chromophore of dyer's broom, whereas luteolin, a minor component of the dye, was not determined in the woollen extract.
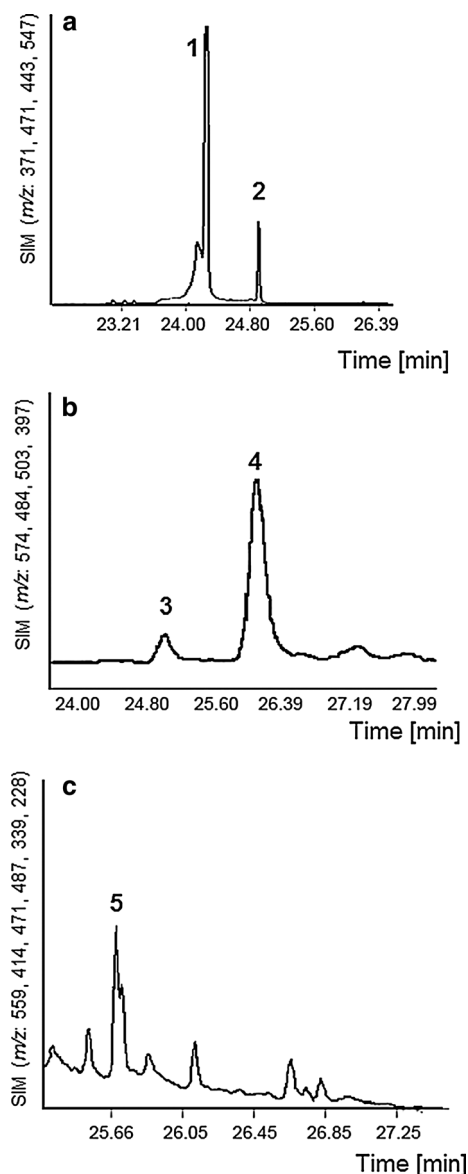
In the sample dyed with madder, the principal components alizarin and purpurin have been easily detected (peaks 12 and 13) together with two other anthraquinones (peaks 10 and 11) recognized thanks to comparison with the mass spectra library. All chromophores detected in wool samples were in their per-TMS form with exception of rhamnetin which was found also in a partially silylated form (peak 6).

The ease of detection of the main markers contained in the samples of the dyed wool is dramatically reduced with
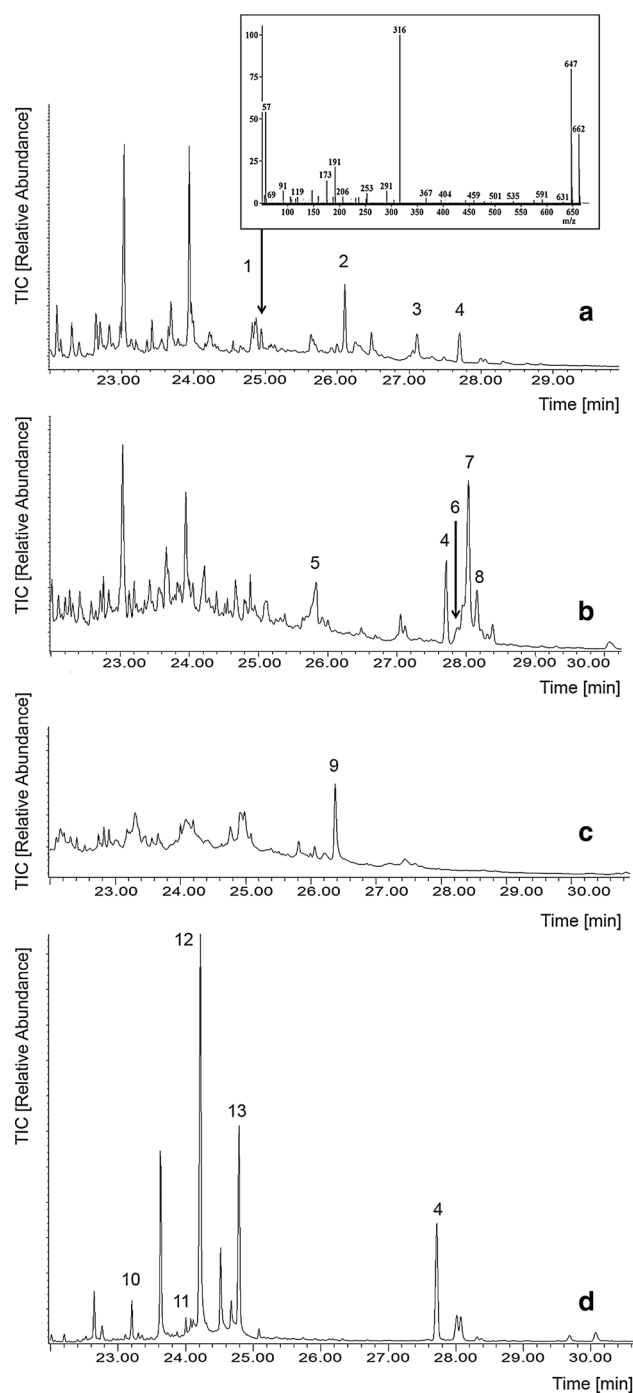
Fig. 8 Chromatograms of the reference woollen dyed samples: old fustic (**a**), Persian berries (**b**), dyer's broom (**c**) madder (**d**). *1* Maclurin per-TMS; *2* morin per-TMS; *3* kaempferol per-TMS; *4* cholesterol TMS ether; *5* emodin per-TMS; *6* rhamnetin tri-TMS; *7* quercetin per-TMS; *8* rhamnetin per-TMS; *9* genistein per-TMS; *10* 1,6-dihydroxy-2-methylanthraquinone, di-TMS; *11* 1,8-dihydroxyanthraquinone; *12* alizarin per-TMS; *13* purpurin per-TMS

Fig. 9 SIM analysis of historical wool samples (**a**). **b** CC 11 sample; **c** CC9 sample. *1* Alizarin per-TMS, *2* purpurin per-TMS, *3* brazilin per-TMS, *4* brazilin 3-TMS, *5* genistein per-TMS

can be normally sampled; the historical samples; in addition, may contain a diminished amount of colouring matter as a result of their aging.

For the analysis of historical samples, therefore, the single ion monitoring (SIM) mode is needed and TIC chromatograms can be used for a preliminary recognition, also using the *"extract ions"* function supplied by the software of the instrument.

The parameters to be used for SIM acquisition were chosen on the basis of the most abundant silylated derivatives of chromophores and on their mass spectra; the retention times of chromophores detected in SIM mode

the decrease of the sample size. This is a very important aspect in the analysis of historical textiles from which only very few milligrams of material (not more than 2 or 3 mg)

were considered acceptable within variation of 2 % of the respective retention time in TIC mode. The expanded significant portions of the SIM chromatograms are shown in Fig. 9.

As hoped, TIC chromatograms of the historical tapestry samples provided a preliminary indication about target molecules of the dyestuff present. Juglone, a naphthoquinone characteristic from plants in the *Juglandaceae* family [2], was clearly recognized in the TIC chromatograms of sample CC11. In addition, the *m/z* 371 extract ion, characteristic of alizarin, indicated the use of madder in the coloration of this sample, also confirmed by the SIM chromatogram (Fig. 9a), where purpurin could be detected, in addition to alizarin. For checking the eventual presence of additional chromophores related to different dye sources other SIM acquisitions have been performed, and one of these runs allowed to detect brazilin, in two different silylated forms (Fig. 9b). The colour of sample CC11, therefore, derives from a dyestuff mixture containing madder, brazilwood and a source of juglone. The use of multiple dyes is not an unusual event in the traditional dyeing technique, as confirmed by the studies on historical samples and by the traditional recipes reported in the literature [1, 2].

In the TIC chromatogram of sample CC6 the *m/z* 414 extract ion was detected, characteristic of the mass spectra of genistein. SIM analysis of the latter, shown in Fig. 9c, has confirmed the presence of the genistein, characteristic of Dyer's broom; no other chromophore has been found in the sample and therefore Dyer's broom is the only dyestuff recognized in this sample.

Conclusions

GC–MS was applied to the analysis of target molecules contained in different plant extracts. The derivatization procedure for the complete silylation of the analytes contained in plants extracts was optimized and the chromatographic conditions established for recognition of the characteristic dyes of each plant extract. It was possible to detect all the main marker molecules contained in each of the considered dyestuff, as well as many other natural components, and a data set for the identification of dyes in plant extracts and in historical textiles have been obtained.

Several constituents of plant extracts such as organic acids, oils and sugars hydrolysed during the extraction process could be also recognized with the same chromatographic run, without interfering with the separation and identification of the species relevant for dyeing purposes. The GC analytical method here proposed may be therefore considered convenient for the quick assessment and the quality control of natural extracts. Among the limitations of the technique, the inability to detect the high molecular weight components must be underlined. This prevents the use of GC–MS for recognition of any glycosylated form eventually survived after extraction from fibres or from plants. For the same reasons, the analysis of other dyes such as cochineal, commonly described in historical dyeing recipes, is prevented.

On the other hand, the advantages of GC–MS are well known: good reproducibility and repeatability of analysis, availability of extended data libraries for recognition of unknown compounds (e.g. the degradation products), and possibility of simultaneous detection of molecules into complex matrices with a single run.

Application of the GC method for analysis of flavones, flavonols and isoflavones gives satisfactory results: flavonoids aglycones are easily recognizable thanks to the complete separation occurring between them and with other natural components contained in plant (such as glycosides, fatty acids and other organic acids).

Neoflavonoids were recognized in the two most important dyestuff sources: haematoxylin, the principal marker of *Haematoxylum campechianum*, was detected in the extract of this plant, as well as in the *Caesalpinia echinata* extract, although in the latter the chromophore is present as a minor component and in very low amount.

Brazilin, the principal marker of *Caesalpinia echinata*, turns out to be a principal component of this extract; however, its keto-form brazilein is not present at detectable levels in the plant extract here considered. Many parts of plants belonging to the *Caesalpiniae* species have a complex composition that includes terpenoids, protosappanins, many phenolic compounds and naphthoquinones. These compounds do not appear in the extract from the wood and, therefore, they do not interfere with the detection of brazilin.

GC separation and detection of anthraquinones from *Rubiacea* and *Polygonaceae* species has been already reported [10, 25], therefore it is not surprising the efficacy of the analysis performed in this work on the madder. On the contrary, no information was available about the application of GC to anthraquinones from *Rahmnacea* species, and the results of the analysis we have obtained on Alder Buckthorn are most encouraging.

This suggests that the GC method is successfully applicable to anthraquinones derivatives formed either through the succinyl benzoic acid pathway, from which anthraquinones of *Rubiacea* and *Polygonaceae* species originate, or through the polyketide pathway that produces anthraquinones and anthrones with both rings hydroxylated, as those in the *Rhamnaceae* species.

GC–MS analysis can be similarly successfully applied for determination of tannins. No overlap is present between the different markers, and sources of condensed tannins and hydrolysable tannins can be distinguished. The

condensed tannins are characterized by the presence of cat-echin and epicatechin, as it occurs in the *Acacia catechu* extract, while the recognition of high levels of ellagic acids is obtained in the analysis of heartwoods containing hydro-lysed tannins, such as in the extracts of *Terminalia chebula* and *Castanea sativa*. Ellagic acid was detected also in the gallnuts extract, even if it is present there in low amount.

Considering the large number of tannin plants used for dyeing and for leather tanning, it would be useful to examine a wider range of samples extracted from different sources in order to assess if the determination of the rela-tive abundances among the principal characteristic compo-nents of each plant may discriminate the plants themselves.

GC analysis was finally tested on woollen references dyed according to traditional recipes, and on historical wool samples taken from a tapestry of sixteenth century. The chromophores contained in Persian berries, dyer's broom, old fustic and madder, used for dyeing the wool reference samples, were easily recognized. The capability of SIM mode for detection of chromophores in historical samples was used to overcome the difficulties due to the sample size limitations and consequent low amounts of colouring matter. The SIM mode, through the detection of the principal ions contained in EI spectra of chromophores, allowed to trace the dyestuff used for some of the colours in an historical tapestry.

The results discussed in this study permit to consider the GC–MS method as a useful analytical technique for approaching archaeometric problems, and support its appli-cation to the analysis of natural dyes in works of art and in historical objects.

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