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Article

Chromatographic and spectroscopic identification and recognition of natural dyes, uncommon dyestuff components and mordants found in the 16th century carpet

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Abstract: A multi-tool analytical practice was used for characterization of 16th century carpet manufactured in Cairo. Mild extraction method with hydrofluoric acid enabled isolation of intact flavonoids and their glycosides, anthraquinones, tannins and indigoids from fibre samples. High-performance liquid chromatography coupled to spectroscopic and mass spectrometric detectors was used for identification of natural dyes present in the historical samples. Weld, young fustic and brazilwood were identified as the dye sources in yellow thread samples. Red fibres have been colored with lac dye, whereas green fibre shades were obtained with indigo and weld. Tannin-containing plant material in combination with indigo and weld were used to obtain brown hue of thread. Four uncommon and thus-far unknown dye components were also found in the historical samples. These compounds probably represent unique fingerprint of dyed threads from this region. Scanning electron microscopy with energy-dispersive X-ray detector (SEM-EDS) and Fourier transformation infrared spectroscopy (FT-IR) were used for identification and characterization of substrates and mordants present in the historical carpet. Carbon and oxygen were detected in large quantities as a part of the wool protein. The presence of aluminum, iron and calcium indicated their usage as mordants. FT-IR analysis showed bands characteristic to woolen fibres and SEM micrographs definite structure of wool.

Keywords: natural dyes; flavonoids; flavone glycosides; anthraquinones; extraction procedure; liquid chromatography mass spectrometry.

1. Introduction

Scientific analysis of objects of artistic and historic significance is a key to reconstructing their story and elucidating the circumstances under which they have been created. Investigation of origin, nature and chemical behavior of the colored materials used in the production of historical artefacts may shed new light on their original color and appearance [1]. Knowledge of historical artworks components essential for the documentation of their authenticity, requires major breakthroughs in interdisciplinary collaborations between archaeologists and analytical chemists [2]. For this reason, many analytical techniques have been employed to the investigation of natural dyestuffs. The identification of colorants in artworks and objects of historical value poses a whole set of analytical challenges due to the wide range of possible dye source and the vast number of chemical classes they belong to, the small amount of sample available for analysis, and the low amount of colored compounds often present at trace levels [3]. Until the mid-19th century, the only sources of dyes for textiles and other fibres were natural materials of vegetal or animal origin. In general, all shades of

color were made from combinations of the primary colors: blue, red, and yellow. Exact identification of coloring substances in biological sources gave us information necessary to determine origin of dyestuffs used to create the object. This data with addition information about metal ions (mordant type dyes) and type of textile raw material used to produce artefact can help in determining how, when and where these works of arts were made.

Extraction of dyes from a textile fibres is usually carried out with boiling hydrochloric acid - methanol mixture. This procedure allows efficient isolation of flavonoids and anthraquinone dyestuffs from textile fibres but causes hydrolysis of theirs glycosidic forms to parent aglycones. Moreover, the use of hydrochloric acid is ineffective for extraction of indigotin and indirubin [4]. For this reason, milder extraction methods based on the use of EDTA, citric, tartaric formic or hydrofluoric acids are currently tested [5-8]. Identification of these components in complex mixtures requires sensitive and selective analytical techniques. Liquid chromatography coupled to spectrophotometric and mass-spectrometric detectors (LC-UV-Vis-MS) has proven to be a useful tool for analyzing works of art, especially those containing natural organic dyestuffs.

Historical carpet originating from Turkish workshop active in the second half of 16th century in Cairo and preserved nowadays in National Museum in Cracow, Poland, was comprehensively studied in the present work. Twelve fibre samples were analyzed in order to identify the natural dyes and mordants used for their manufacture. Mild extraction method with hydrofluoric acid have been optimized for dyestuffs isolation from the wool samples. The dyes were identified by high-performance liquid chromatography-mass spectrometry with atmospheric pressure electrospray ionization in negative mode LC-ESI(-)-MS and confirmed by quadrupole time-of-flight (QTOF) mass spectrometry. Twenty six dyestuffs were detected and recognized or tentatively characterized, of which four compounds were not described before. LC-MS technique was also applied to analyze reference dyestuff in extracts of weld (*Reseda luteola* L.), lac dye (*Kerria lacca*), and indigo (*Isatis tinctoria*) in order to provide indications of the structures of coloring substances which were detected in the historical samples but are not commercially available in pure form. Mordant ions identification was performed by scanning electron microscopy with energy-dispersive X-ray detectors (SEM-EDS). Fourier transform infrared spectroscopy (FT-IR) was utilized for substrate characterization. The results reported in the present paper enabled full dye fingerprints in fibre samples.

2. Materials and Methods

2.1. Chemicals and materials

Acetonitrile and methanol used as mobile phase components were of HPLC grade and were purchased from Merck (Darmstadt, Germany). Hydrofluoric acid (48% in water) was purchased from Sigma-Aldrich (Steinheim, Germany). Dimethyl sulfoxide (DMSO, ACS grade) was obtained from Merck KGaA (Darmstadt, Germany). Synthetic dyestuffs: luteolin, apigenin, fistein and luteolin 7-O-β-D-glucoside of HPLC purity were purchased from Sigma-Aldrich. Raw dyestuff materials: weld (Reseda luteola L.), lac dye (Kerria lacca), and indigo (Isatis tinctoria) were obtained from Kremer Pigmente (Aichstetten, Germany) in dried form. The dyestuff materials were homogenized prior to analysis. All aqueous solutions were prepared using deionized Milli Q water.

2.2. Origin of textile fibre samples

The samples of textile fibres were collected from historical carpet exhibited in the National Museum in Cracow (Poland). The carpet with Chintamani motifs is unique because it is one of the largest preserved carpet in the world with surface area of almost 40 square meters, 1063 cm long and 372 cm wide (Figure 1). Origin of the rug was attributed to a Turkish workshop active in the second half of the 16th century in Cairo basing on composition analysis, identification of motifs and the technique used. According to the church tradition, the carpet was donated by Stanislaw Jablonowski, the colonel of Polish King Jan Sobieski (1629-1696), after his return from the victorious Viennese battle (1683). In 1901 it was transferred from the Corpus Christi Church in Cracow to the collection of National Museum. The carpet survived in eight parts, what cannot be explained merely by breaking

the structure of the warp, weft, and Persian knots resulting from damage during usage. Some regular line intersections indicate purposeful extraction of parts perhaps for the needs of the user or collectors of art works. One of the detached fragments is now in the collection of the Munich Museum of Ethnology.

Twelve fibres samples were collected from different parts of the carpet and referred as: F1, F2, F3 and F4 (yellowish of various shades), F5, F6 (light and dark reds), F7 (navy blue), F8, F9 and F10 (greens of different shades), F11 (brown), and F12 (beige).

Figure 1. Fragment of historical carpet with fibre sampling location (National Museum in Cracow, collection MNK XIX-8950).

2.3. Extraction of dyes from threads

Dyes were extracted from the thread samples (estimated weigh 0.2 mg) in an ultrasonic bath for 1 hour (4 x 15 min) at temperature not exceeding 40 °C using 500 µL of solution containing 0.4 M hydrofluoric acid/methanol/acetonitrile/DMSO (2:1:1:1, v/v). The mixtures were centrifuged at 9000 rpm for 5 min and the supernatants were evaporated almost to dryness under a stream of nitrogen. The residues were taken up in 300 µL ACN/MeOH/DMSO, (1:1:1, v/v), out of which 2 µL were injected into the HPLC column. The raw materials used as reference for the analysis of the historical textile samples were also extracted according to the HF procedure. Synthetic standards used for identification purposes were dissolved in ACN/MeOH/DMSO (1:1:1, v/v) mixture.

2.4. Equipment

The morphology of samples was studied with FEI Quanta FEG 250 Scanning Electron Microscope (SEM) operated with a secondary electron detector in a high vacuum mode at accelerating voltage 10-20 kV. The identification of elements was performed using energy dispersive X-ray spectroscopy (SEM-EDS) on EDAX Genesis APEX 2i with ApolloX SDD spectrometer at accelerating voltage 20 kV.

Infrared transmission spectra (FTIR) were recorded with Nicolet iS50 FT-IR spectrometer equipped with the Specac Quest single-reflection diamond attenuated total reflectance (ATR) accessory. Spectral analysis was controlled by OMNIC software package.

Analyses were performed using liquid chromatograph series 1290 (Agilent Technology, Waldbronn, Germany) consisting of binary pump G4220A, autosampler G4226A, thermostated column compartment G1316C, diode-array detector G1315C, and triple quadrupole mass spectrometer G6470 with AJS electrospray ionization source. The chromatographic system was controlled with Agilent MassHunter software. The components of the extracts were separated on C-18 reversed-phase column. The analytes were monitored with diode array detector and mass

spectrometer connected in-line and characterized with their retention times, UV-vis and mass spectra. In order to ensure universal elution conditions for chemically different compounds a wide gradient of methanol/acetonitrile and water has been used. The structures of identified dyes were confirmed by LC-ESI(-)-QTOF analysis using Agilent 1290 LC system coupled to Agilent quadrupole time-of-flight (QTOF) mass spectrometer G6540 operated in negative ionization scan mode under the same chromatographic conditions. The parameters of the optimized spectrochromatographic analysis are presented in Table S1.

3. Results and Discussion

3.1. Microscopic and spectroscopic studies

3.1.1. Surface morphology

Surface profiles, nature, homogeneity and microstructure of the samples of the historical fibres were analyzed using scanning electron microscope. SEM micrographs of fibre surfaces display cylindrical shape and nodular thickening across their length, all of which are characteristics for scale structure of wool (Figure 2a-c). In some cases roughened surfaces were observed with damage of this scale structure due to natural ageing (Figure S1). Some of the samples were more degraded since they have greater number of fractured fibrils. The diameters of the wool fibres ranged from 20 to 40 μm.

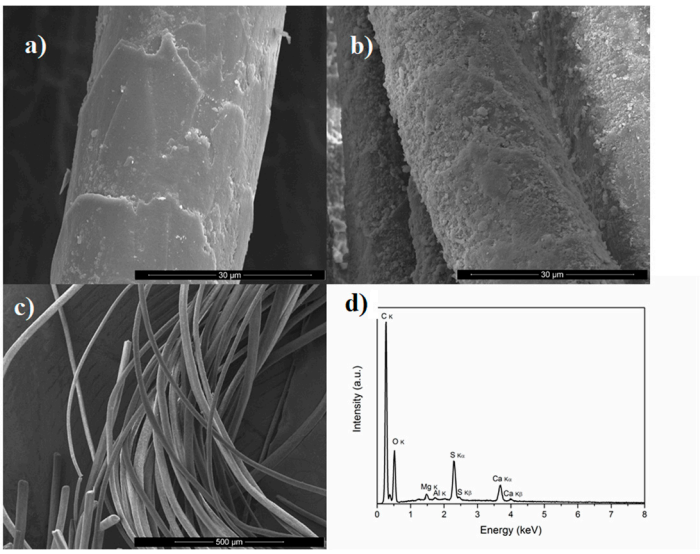

Figure 2. SEM micrographs of: (a) fibre F1 (magnitude 100x), (b) fibre F11 (magnitude 100x), (c) fibre F1 (magnitude 2500x), (d) SEM-EDS spectrum of fibre F1.

3.1.2. FTIR analysis

Infrared is a highly suitable technique for fibres characterization and enables distinguish their origin [9]. The IR spectra of all fibres (Figure S2) showed broad stretching band of amino -NH and phenolic -OH groups at 3275 cm^{-1}. Adsorption at 2925 and 2880 cm^{-1} was due to the C-H asymmetric stretching of aliphatic carbon compounds. The IR peak at 1225 cm^{-1} was due to C-N stretching. Weak adsorption band near 1040 cm^{-1} was attributed to presence of ether linkages. Amide I (1700–1600 cm^{-1}), and amide II (1550-1500 cm^{-1}) bands, which are characteristics of the protein structure, were also observed in the IR spectra of the all investigated samples. Thus, the samples were unequivocally animal fibres [10]. Moreover, observation of the 640-650 cm^{-1} and 590-525 cm^{-1} bands, designated to ν (C-S) stretching vibrations indicates that threads samples are woolen fibres [11].

3.1.3. SEM-EDS analysis

SEM-EDS was employed to identify the mordant metals used during dyeing of the historical threads. A typical EDS spectrum of textile specimens is given in Figure 2d. Two elements, i.e. carbon and oxygen, arising from wool proteins were detected in large quantities (Table 1). The presence of Al, Fe, S, Ca, Mg, Si and trace amount of copper were also found. Aluminum and iron probably originate from a mordant essential to obtain fast colors. While aluminum salts do not change the colors of dyed textiles, iron and copper salts cause darkening of yellow and red mordant dyes and tannins affecting final color of the textile fragments during dyeing process [12]. Calcium, Si and Mg might originate from the contaminants during storage and utilization of the carpet, thus it is not possible to conclude if they are the components of the mordants. The presence of sulphur is not surprising since this element is found in animal fibres. Chromium and K were not detected in any of the investigated samples, although their salts could have been used as mordants in the past. The investigated samples did not differ significantly in respect of elemental composition although relative amounts of particular elements were variant. The brown fibre F11 was probably covered by greatest amount of iron and calcium salts as can be seen in the Figure 2d and Table 1. EDS spectrum of this sample exhibit also the highest content of sulphur and silica.

Table 1. Composition of elements (in atomic % [a]) based on EDS analysis.

Fibre No. / Element	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
C	70	64	76	67	72	66	71	64	62	65	58	69
O	27	33	22	31	27	32	27	34	36	33	33	26
Al	0.5	0.6	0.2	0.1	0.1	0.3	0.1	0.3	0.3	0.3	0.6	0.5
Si	0.2	0.3	-	0.1	-	0.1	0.1	0.4	0.4	0.1	0.9	0.6
S	0.8	0.5	1.0	0.7	0.7	0.7	0.6	0.5	0.6	0.5	3.5	2.7
Ca	0.5	0.5	0.4	0.4	1.0	0.4	0.5	0.3	0.5	0.4	2.1	0.6
Fe	<0.1	<0.1	-	0.15	0.2	0.1	<0.1	<0.1	<0.1	<0.1	1.6	0.1
Mg	0.2	0.2	0.2	0.1	<0.1	<0.1	0.1	<0.1	0.1	<0.1	0.2	0.3
Na	-	0.5	-	-	-	-	-	-	-	-	-	0.5
traces [b]	P, Cu	P	Cu	Cu	Cu	Cu	-	Cu	P, Cu	P	-	Cu

[a] Uncertainty of oxygen and carbon is +/- 3%, for other elements +/- 0.1%; [b] elements detected below 0.1 % have been labelled as "traces".

3.2. LC-MS analysis

The identification of coloring compounds was performed by comparison of their retention times, UV and mass spectra in the negative ionization mode (ESI(-)-MS) to these obtained for the compounds found in weld (*Reseda luteola* L.), lac dye (*Kerria lacca*), and indigo (*Isatis tinctoria*) extracts and standards of flavonoids under the same chromatographic conditions (see experimental section). Identification of standardless and unknown coloring substances was supported by high-resolution QTOF spectra.

3.2.1. Yellow fibres

Four historical fibres possess yellow hue. Figure 3 shows the chromatograms obtained for extracts of fibres: a) F1, b) F2, c) F3, and d) F4. The retention times, maximum absorbance wavelengths (λ_{max}), computed elemental compositions, molecular ions, main fragment ions and proposed formulae of the compounds are summarized in Table 2. The chemical structures and MS spectra of all the identified compounds are presented in supplementary information (Figures S3-S32).

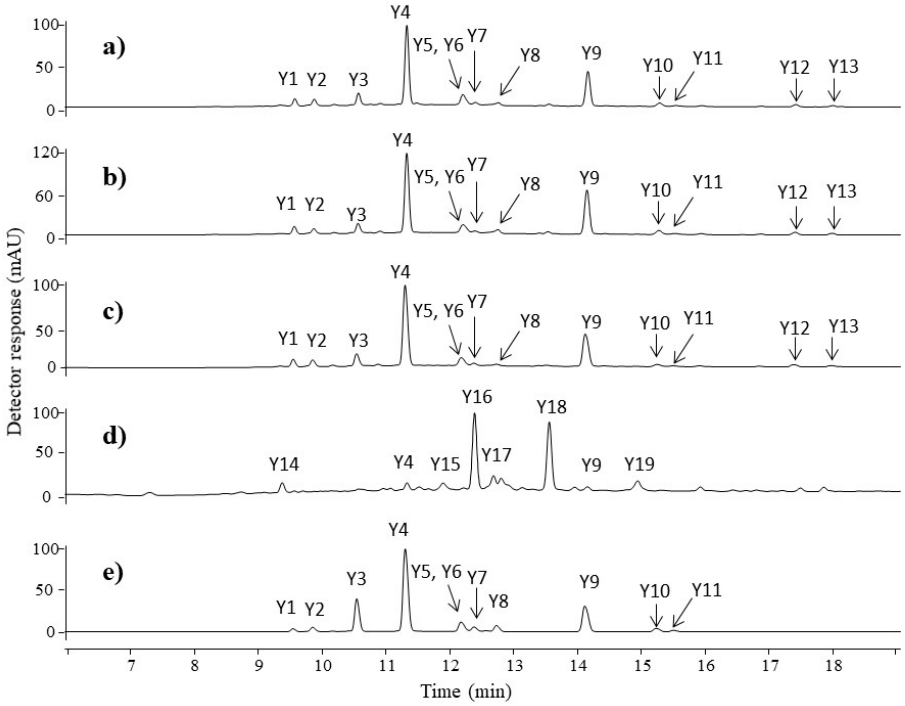

Figure 3. HPLC-DAD chromatograms (λ=350 nm) of yellow extracts taken from: (a) fibre F1, (b) fibre F2, (c) fibre F3, (d) fibre F4, and (e) weld raw source. For chromatographic conditions see Table S1.

The chromatographic profiles obtained for F1, F2 and F3 samples are quite similar and comparable to chromatogram of weld extract (Figure 3e). Eight flavone-glucosides and five aglycons could be identified in yellow fibres. First compound (Y1) eluting at 9.5 min was recognized as apigenin-*C*-diglucoside. It showed pseudo-molecular ion $[M-H]^-$ at m/z 593 and main fragmentation patterns at m/z 503 $[M-H-90]^-$, m/z 473 $[M-H-120]^-$, and m/z 575 $[M-H-18]^-$. Losses of 120 and 90 units correspond to cross-ring cleavage in sugar moiety characteristic for *C*-glycosides, whereas ion at m/z 575 is formed by neutral loss of H_2O molecule. The hypothesis was confirmed by ESI(-)-QTOF product ion mass spectrum in which the peak $[M-H]^-$ was observed at m/z 593.1513 (corresponding to the elemental composition of $C_{27}H_{30}O_{15}$, mass diff. -0.17 ppm). Compound Y2 with pseudo-molecular ion $[M-H]^-$ at m/z 609 fragmented to $[M-H-162]^-$ ion at m/z 447 and $[M-H-162-162]^-$ ion at m/z 285 (aglycone). The fragment ions correspond to loss of one glucose and two glucose moiety from luteolin glucoside. This compound was assigned as luteolin-*O*-diglucoside.

Table 2. Spectrochromatographic data of the components extracted from all historical fibres.

Peak No.	t_R (min)	$[M-H]^-$, *m/z*		Fragment ions (m/z)	Elemental composition	Diff (ppm)	Proposed identification	χ_{max} (nm)
		Nominal	Highly					
Y1	9.6	593	593.1513	503, 575, 473, 383	$C_{27}H_{30}O_{15}$	-0.17	apigenin-*C*-diglucoside	272, 335
Y2	9.9	609	609.1442	447, 285	$C_{27}H_{30}O_{16}$	3.12	luteolin-*O*-diglucoside	268, 336
Y3	10.6	609	609.1462	447, 285	$C_{27}H_{30}O_{16}$	-0.16	luteolin-3,7′-*O*-diglucoside	268, 341
Y4	11.3	447	447.0923	285, 284	$C_{21}H_{20}O_{11}$	2,24	luteolin-7-*O*-glucoside	268, 349
Y5	12.2	447	447.0932	285	$C_{21}H_{20}O_{11}$	0.24	luteolin-*O*-glucoside	268, 337
Y6	12.2	431	431,0989	311, 269, 268	$C_{21}H_{20}O_{10}$	-1.16	apigenin-7-*O*-glucoside	266, 348
Y7	12.4	461	461,1072	341, 299, 284, 283	$C_{22}H_{22}O_{11}$	3.68	chryoseriol-*O*-glucoside	266, 348
Y8	12.8	447	447.0936	285	$C_{21}H_{20}O_{11}$	-0.67	luteolin-4′-*O*-glucoside	268, 342
Y9	14.2	285	285.0407	257, 217, 199, 175, 151, 133	$C_{15}H_{10}O_6$	-1.05	luteolin	255, 349
Y10	15.3	269	269.0454	225, 151, 117	$C_{15}H_{10}O_5$	0.37	apigenin	267, 337

Y11	15.5	299	299.0563	284, 256	$C_{16}H_{12}O_6$	-0.67	chryoseriol	266, 347
Y12	17.4	313	313.0349	285, 243, 201, 179, 133	$C_{16}H_{10}O_7$	1.60	luteolin derivative	248, 346
Y13	18.0	313	313.0342	285, 243, 201, 179, 133	$C_{16}H_{10}O_7$	3.83	luteolin derivative	242, 346
Y14	9.4	349	349.0028	371, 338, 269, 225, 213, 177, 165, 149, 135, 121	-	-	unknown	261, 392
Y15	12.0	243	243.0294	215, 199, 187, 175, 145, 113	-	-	type C	308, 336
Y16	12.4	269	269.0448	241, 225,185, 135, 133	$C_{15}H_{10}O_5$	2.60	sulfuretin isomer	316, 343
Y17	12.7	285	285.0407	241, 229, 149, 135, 121	$C_{15}H_{10}O_6$	-1.05	fistein	320, 360
Y18	13.5	269	269.0459	241, 225, 213, 195, 135	$C_{15}H_{10}O_5$	-1.47	sulfuretin	256, 396
Y19	14.9	314	314.0302	267, 239, 217, 199, 163, 135	-	-	unknown	292, 342
R1	8.5	522	522.0656	478, 434	$C_{25}H_{17}NO_{12}$	4.02	xantholaccaic acid C	293, 425
R2	9.0	538	538.0626	520, 494,476, 450, 432	$C_{25}H_{17}NO_{13}$	0.19	laccaic acid C	288 ,490
R3	9.5	494	494.0723	476, 450, 432, 406, 388, 378	$C_{24}H_{17}NO_{11}$	1.21	laccaic acid E	288, 490
R4	11.0	552	552.0769	534, 508, 490, 464, 446	$C_{26}H_{19}NO_{13}$	2.54	derivative of laccaic acid A	285, 504
R5	11.1	520	520.0854	502, 476, 458, 432, 414	$C_{26}H_{19}NO_{11}$	5.96	xantholaccaic acid A	294, 430
R6	11.3	536	536.0836	518, 492, 474, 448, 430, 420	$C_{26}H_{19}NO_{12}$	-0.37	laccaic acid A	288, 490
R7	11.3	495	495.0568	477, 451, 433,407, 389	$C_{24}H_{16}NO_{12}$	0.2	laccaic acid B	288,490
R8	12.5	606	606.1184	562, 518	-	-	unknown	288, 492
B1	18.1	261	261.0665	233, 217	$C_{16}H_{10}N_2O_2$	1.53	indigotin	288, 620
B2	19.2	261	261.0669	233, 217	$C_{16}H_{10}N_2O_2$	0	indirubin	290, 550
BR1	11.5	301	300.9992	284, 257, 229	$C_{14}H_8O_8$	2.3	ellagic acid	255, 355

Compound Y3 with pseudo-molecular ion at m/z 609 fragmented by the loss of two glucose moiety leading to ions at m/z 447 and m/z 285 (aglycone). High intensity of $[M-H-162]^-$ ion suggests that sugar residues are bounded at different aglycone positions. The compound was identified as luteolin-3,7′-*O*-diglucoside basing on UV spectra and data available in the literature [13,14]. Compound Y4 as well as compound Y8 were attributed to isomers of luteolin-*O*-glucoside. Both compounds have a pseudo-molecular ion at 447 m/z which losses glucose residue (162 Da) leading to aglycone ion at m/z 285. An ion at 284 m/z $(aglycone-H)^{\bullet-}$ observed in product ion m/z 285 mass spectrum of compound Y4 is known in the literature as a marker for distinguishing luteolin-7-*O*-glucoside (Y4) and luteolin-4′-*O*-glucoside (Y8) [15]. The identification of luteolin-7-*O*-glucoside was straightforward as this compounds is available in pure form. The chromatographic peak at retention time 12.2 min contains two co-eluting compounds Y5 and Y6. First of them was considered as luteolin-*O*-glucoside with pseudo-molecular ion $[M-H]^-$ at m/z 447 and fragment ion at m/z 285 (aglycone). The second component (Y6) was attributed to apigenin-7-*O*-glucoside with the parent ion $[M-H]^-$ at m/z 431 and daughter ions at m/z 311, 269 and 268. Signal at m/z 311 ($^{0,2}X^-$ ion resulting from the loss of 120 Da) in MS/MS spectra of compound Y6 was attributed to the 0,2-cleavage of the glucose moiety which is common for flavone-7-*O*-glucoside [15]. Compound Y7 eluting at 12.4 min with pseudo-molecular ion at m/z 461 and fragment ion at 299 m/z, formed after loss of glucose moiety, was attributed to the chrysoeriol glucoside. Compounds Y9, Y10 and Y11 were identified as luteolin, apigenin and chrysoeriol, respectively. Mass spectra of luteolin showed characteristic ions at m/z: 257 $[M-H-CO]^-$, 217 $[M-H-C_3O_2]^-$, 199 $[M-H-C_2H_2O-CO_2]^-$, 175 $[M-H-C_3O_2-C_2H_2O]^-$ as well as ions corresponding to retro-Diels-Alder (RDA) fragmentation of flavone molecule which gave rise to two species: $^{1,3}B^-$ ion at m/z 133 and $^{1,3}A^-$ ion at m/z 151 [16]. Apigenin showed similar RDA fragmentation pathways leading to $^{1,3}B^-$ ion at m/z 117 and $^{1,3}A^-$ ion at m/z 151. Identification of these compounds was straightforward as luteolin and apigenin standards are available. Chryoseriol fragmented by loss of $CH_3^\bullet$ unit to ion at m/z 284 which is characteristic for methoxy derivative of flavones, and subsequently by the neutral loss of CO molecule yielding a product ion at m/z 256. The chromatographic profiles of fibres F1, F2 and F3 may suggest that weld was the source of the yellow

dyes. Two additionally peaks (Y12 and Y13) were detected in F1, F2 and F3 fibres extracts. Mass spectra of both compounds Y12 (RT 17.4 min) and Y13 (RT 18.00 min) showed pseudo-molecular ion at m/z 313 and main fragment ions at m/z: 285 $[M-H-CO]^-$, 243 $[M-H-CO-C_2H_2O]^-$, 201 $[M-H-CO-2C_2H_2O]^-$, 179 $[M-H-134]^-$, and 133 $[M-H-180]^-$, respectively. These compounds are not present in weld extract. Signals at 179 and 133 m/z correspond to ions $^{1,3}A^-$ and $^{1,3}B^-$ formed during RDA fragmentation of flavone molecule (Figure 4). Unique fragment ion at m/z 133 is similar to $^{1,3}B$ fragment ion of luteolin, whereas daughter ion at 179 m/z differs by 28 atomic mass units from the luteolin $^{1,3}A^-$ ion. This difference suggests that in A ring of compounds Y12 and Y13 additional substituent containing carbon and oxygen elements is present, i.e. formyl group. Different retention times of compounds Y12 and Y13 imply this substituent is attached to different positions at the aglycone, although it is not possible to conclude the exact location. This hypothesis was confirmed by ESI(-)-QTOF product ions mass spectra in which the pseudo-molecular ions of compounds Y12 and Y13 corresponding to the elemental composition of $C_{16}H_{10}O_7$were observed at m/z 313.0349 (mass diff. 1.6 ppm) and 313.0342 (mass diff. 3.8 ppm). To the best of our knowledge, these compounds were not described in the literature before.

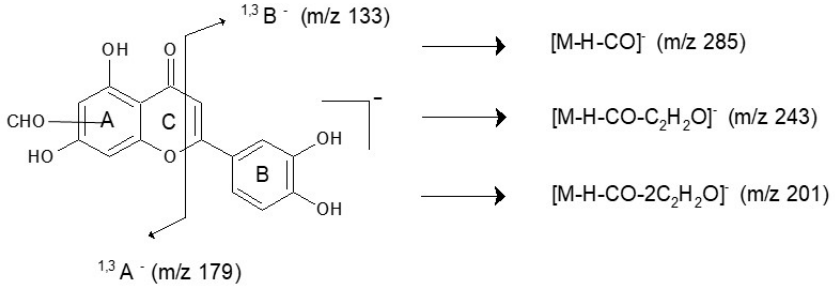

Figure 4. Fragmentation pathway of flavone molecules Y12 and Y13.

The UV chromatogram of the orange-yellow fibre F4 extract showed two main peaks at 12.4 min and 13.6 min. Peak eluted at 13.6 min (Y18) with pseudo-molecular ion at m/z 269 gave fragment ions at m/z: 241 $[M-H-CO]^-$, 225 $[M-H-CO_2]^-$, 213 $[M-H-2CO]^-$, 195 $[M-H-2CO-H_2O]^-$, 135, and 133. Ions at m/z 135 and 133 were formed from the A and B rings of aurone. UV spectrum of this substance had λ_{max} at 256, 275, and 396 nm. The obtained results and literature data allowed us to postulate that detected compound is sulfuretin [17-19]. Peak eluted at 12.4 min, marked as Y16, has also ion $[M-H]^-$ at m/z 269 and fragment ions at m/z 241, 225, 135 and 133, similar to Y18. This compound seems to be an isomer of sulfuretin, however the exact structure is unclear. The peak Y17, appearing at the retention time 12.7 min, with mass peak $[M-H]^-$ at m/z 285 and product ions of aglycone RDA fragmentation at m/z: 241 $[M-H-CO_2]^-$, 229 $[M-H-2CO]^-$, 149, 135, and 121 was identified as fistein. Identification of this compound was straightforward as fistein standard is available. Some other dyestuffs were detected in yellow thread F4. Minor peaks in the chromatogram (Figure 3d) indicated as compounds Y14, Y15 and Y19 showed pseudo-molecular ions $[M-H]^-$ at m/z 349, 243 and 314, respectively. The structures of these compounds remains unknown. The peak labelled as Y15 showed main ion at m/z 243 with fragment ions at m/z: 215 $[M-H-CO]^-$, 199 $[M-H-CO_2]^-$, 187 $[M-H-2CO]^-$, 175, 113. Absorption spectrum showed maxima at 258, 308 and 336 nm. Based on the result obtained and data available in the literature, compound Y15 was identified as the photodegradation product of brazilein, known as a "type C" compound [20,21]. Brazilwood is known for its fast light degradation and type C compound is often used as marker for the identification of the soluble redwoods in samples extracted from historical artworks [22,23]. Despite the fact that sample F4 nowadays presents a yellowish tone, it is thought to have been originally dyed in an orange hue. The presence of sulfuretin, luteolin and type C compound led us to conclusion that the fibre F4 was dyed with young fustic, weld, and brazilwood.

3.2.2. Red fibres

The chromatographic profiles of red fibres extracts (F5 and F6) revealed presence of one major and six minor peaks (Figure 5a-b).

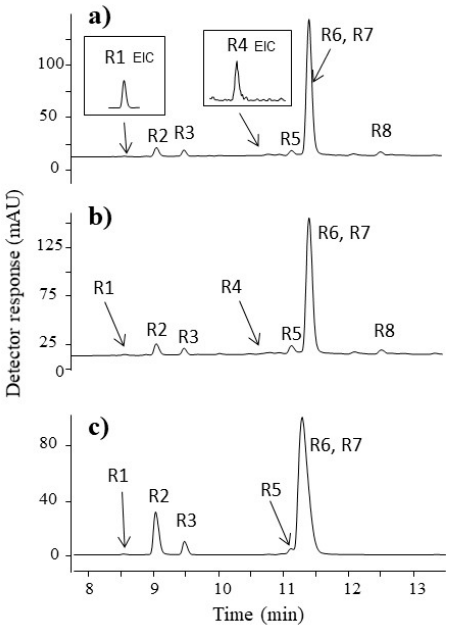

Figure 5. HPLC-DAD chromatograms (λ=350 nm) of red extracts taken from: (a) fibre F5, (b) fibre F6 and (e) lac dye raw source. For chromatographic conditions see Table S1.

Major chromatographic peak seen in UV spectrochromatograms at 11.3 min (R6 and R7) had rather complex mass spectrum. The chromatogram deconvolution algorithm of MassHunter software revealed two co-eluting components. The reconstructed mass spectrum of the first component showed pseudo-molecular ion at m/z 536 and the main product ions at m/z 492 and 448, while pseudo-molecular ion at m/z 495 with two most intensive product ions at m/z 451 and 407 can be seen in reconstructed mass spectrum of the second component (see Table 2). The structure of the first component corresponds to laccaic acid A with fragment ions at m/z: 518 $[M\text{-}H\text{-}H_2O]^-$, 492 $[M\text{-}H\text{-}CO_2]^-$, 474 $[M\text{-}H\text{-}CO_2\text{-}H_2O]^-$, 448 $[M\text{-}H\text{-}2CO_2]^-$, 430 $[M\text{-}H\text{-}2CO_2\text{-}H_2O]^-$, and 420 $[M\text{-}H\text{-}2CO_2\text{-}CO]^-$. Laccaic acid B was assigned to the second component with product ions at m/z: 447 $[M\text{-}H\text{-}H_2O]^-$, 451 $[M\text{-}H\text{-}CO_2]^-$, 433 $[M\text{-}H\text{-}CO_2\text{-}H_2O]^-$, 407 $[M\text{-}H\text{-}2CO_2]^-$, 389 $[M\text{-}H\text{-}2CO_2\text{-}H_2O]^-$. Decarbonylation of one or more of the keto groups is the main fragmentation reaction of anthraquinoids. Successive decarbonylation may lead to fragment ions $[M\text{-}H\text{-}28]^-$, $[M\text{-}H\text{-}56]^-$ and $[M\text{-}H\text{-}84]^-$. Decarboxylation of the carboxylic acid group $[M\text{-}H\text{-}44]^-$ is an characteristic fragmentation pathway for laccaic acids, followed by the loss of water $[M\text{-}H\text{-}62]^-$. Another intensive fragment ions $[M\text{-}H\text{-}88]^-$ and $[M\text{-}H\text{-}106]^-$ correspond to the loss of two CO_2 moles and subsequent loss of water from the fragment ion $[M\text{-}H\text{-}2CO_2\text{-}H_2O]^-$.

Peak R2 had mass ion $[M\text{-}H]^-$ at m/z 538 accompanied by product ions at m/z 520, 494, 476, 450, and m/z 432. The peak at m/z 538 can be attributed to the depronated molecular ion of laccaic acid C with product ions formed by loss of H_2O or CO_2 molecules. The mass peak at m/z 494 (compound R3) accompanied by related peaks at m/z: 476 $[M\text{-}H\text{-}H_2O]^-$, 450 $[M\text{-}H\text{-}CO_2]^-$, 432 $[M\text{-}H\text{-}CO_2\text{-}H_2O]^-$, 406 $[M\text{-}H\text{-}2CO_2]^-$, 388 $[M\text{-}H\text{-}2CO_2\text{-}H_2O]^-$, 378 $[M\text{-}H\text{-}2CO_2\text{-}CO]^-$ proved the presence of laccaic acid E. The chromatographic peak R5 had the signal of the pseudo-molecular ion registered at m/z 520 and fragment ions at m/z 502, 476, 458, 432 and 414, formed by subsequently loss of water and carbon dioxide. Observed signals indicated that the examined extract contain xantholaccaic acid A which is a derivative of laccaic acid A after loss of hydroxyl substituent in the anthraquinone skeleton. These results are in agreement with the data available in the literature [24]. The enlargement of the UV chromatographic trace shows peak of low intensity at retention time 8.5 min (peak R1) eluted before the peak of laccaic acid C. Mass spectrum of this compound revealed the presence of parent ion $[M\text{-}H]^-$ at m/z 522 and daughter ions at m/z 478 $[M\text{-}H\text{-}CO_2]^-$, and m/z 434 $[M\text{-}H\text{-}2CO_2]^-$. Accurate mass

measurement of R1 pseudo-molecular ion (m/z 522.0656, mass diff. 4.02 ppm) and main fragment ions (m/z 478.0762, mass diff. 3.56 ppm and m/z 434.0879, mass diff. 0.46 ppm) allow us to identify the examined compound as the derivative of laccaic acid C formed after loss of hydroxyl group. To our best knowledge, this derivative, named by us xantholaccaic acid C, is so far unknown in the literature. Peak R4, appearing at the retention time 11.0 min, showed a pseudo-molecular ion [M-H]- at m/z 552 that generated fragment ions at m/z 534 [M-H-H_2O]-, m/z 508 [M-H-CO_2]-, m/z 490 [M-H-CO_2-H_2O]-, m/z 464 [M-H-2CO_2]-, m/z 446 [M-H-2CO_2-H_2O]-. This compound was tentatively identified as derivative of laccaic acid A enriched by one additional hydroxyl group in the anthraquinone skeleton. This hypothesis was confirmed by the ESI(-)-QTOF product ion mass spectrum with the pseudo-molecular peak of [M-H]- at m/z 552.0769 corresponding to the elemental composition of $C_{26}H_{19}NO_{13}$ (mass diff. 2.54 ppm) and fragment ion at m/z 534.0675 ($C_{26}H_{17}NO_{12}$, mass diff. 0.56 ppm), although it is not possible to conclude the exact position of this hydroxyl substituent. This compound was detected only in the naturally aged fibre samples but not in lac dye extract. It was probably formed from its precursor, laccaic acid A (compound R6) during dyeing process or as a consequence of ageing. To our best knowledge, this coloring compound was not so far reported in the literature. Comparison of HPLC profiles of red fibre and lac dye extracts (Figure 5c) allow us to conclude that *Kerria lacca* insect has been used in dyeing process of these threads.

3.2.3. Blue and green fibres

LC-ESI(-)-MS of the blue fibre extract (F7) indicated presence of two compounds B1 and B2 (Figure 6a). Compound B1 with pseudo-molecular ion [M-H]- at m/z 261, and two less intensive fragment ions at m/z 233 [M-H-CO]-, and 217 [M-$CONH_2$]- was identified as indigotin. Compound B2, assigned as indirubin, showed the same pseudo-molecular ion ([M-H]- at m/z 261) and similar fragmentation pathway. Fortunately, indigotin and indirubin can be easily differentiated due to their different retention times (18.1 and 19.2 min) and absorption spectra in the visible region. Indigotin has maximum absorbance at 620 nm whereas indirubin at 550 nm. Detection of these coloring substances suggests that indigo was used for dyeing, but it is not possible to determine what kind of plant (*Isatis tinctoria* or *Indigoferia tinctoria*) was used during the dyeing process.

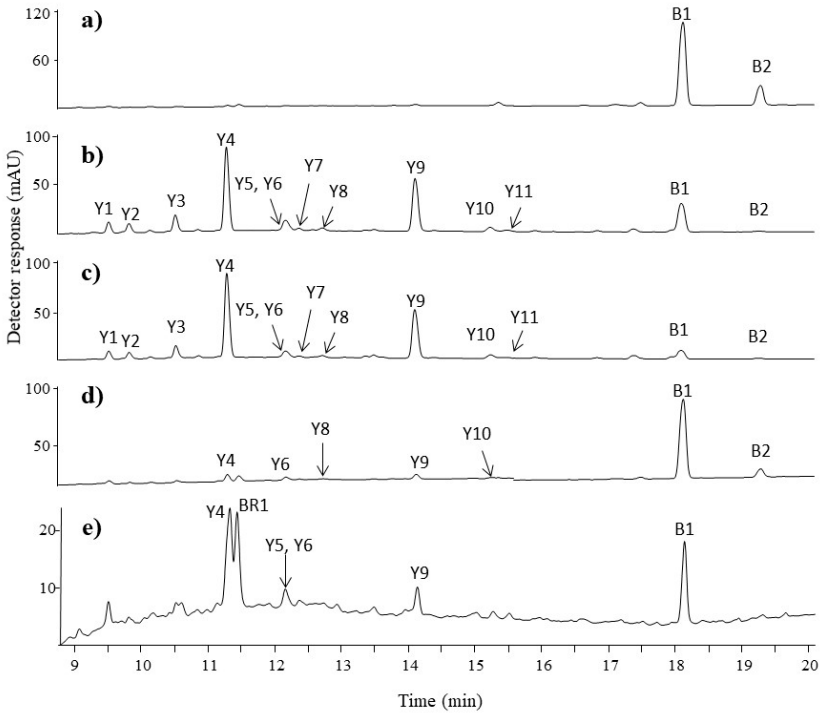

Figure 6. HPLC-DAD chromatograms (λ=350 nm) of blue, green and brown extracts taken from: (a) fibre F7, (b) fibre F8, (c) fibre F9, (d) fibre F10, and (e) fibre F11. For chromatographic conditions see Table S1.

Extracts of three fibres (F8, F9, F10) exhibited green hue. UV chromatograms of F8 and F9 samples were very similar (Figure 6b-c). Eleven flavonoids were detected in these extracts. The presence of luteolin (Y9), apigenin (Y10), chryoseriol (Y11), and its glycosides (see Table 2, compounds Y1-Y8) confirms the use of weld in coloring process. Both vat dye constituents, indigotin and indirubin, were also detected. Due to the fact that natural green dyes are rare, the green hues were usually obtained by sequential dyeing with blue and yellow dyes [25]. It was confirmed by the presence of blue indigoids and yellow flavonoids (luteolin-7-*O*-glucoside, apigenin-7-*O*-glucoside, luteolin-4'-*O*-glucoside, luteolin and apigenin) in all the green fibres extracts. Many historical recipes refer the use of indigo and weld to dye wool fibres in green hues and that combination has already been reported in the literature [23,26]. As expected, the higher amount of indigotin is related to darker green hue of sample F10 in comparison to fibres F8 and F9.

3.2.4. Beige and brown fibres

Fibre designated as F11 has a bronze hue. Brown shades were obtained by the use of three types of dyes. Traces of "luteolin-type" flavonoids were detected in this wool extract: luteolin-7-*O*-glucoside (Y4), luteolin-*O*-glucoside (Y5), apigenin-7-*O*-glucoside (Y6), and luteolin (Y9) (Figure 6e). Dyestuff composition suggest the use of weld, but chrysoeriol was not detected, therefore the use of another luteolin-containing plant may not be excluded [27]. The presence of ellagic acid (BR1), indigotin (B1) and indirubin (B2) indicates the use of a tannin-containing plant material and indigo, either for textile dyeing or for weighting the wool [28]. Tannins in combination with iron mordants have been frequently used in the past to achieve brown or black hue of threads [4,28,29]. This procedure could explain the highest amount of iron detected in the fibre F11 by SEM-EDS (Table 1). Thread F12 probably was not pigmented because even no trace amount of dyes was found in it.

4. Conclusions

Multi-analytical approach combining LC-UV, LC-MS, FT-IR, and SEM-EDS was fundamental to the successful identification and characterization of substrates, mordants and coloring substances present in the historical carpet. SEM micrographs of the fibres display cylindrical shape with nodular thickening across their length which are characteristics for scale structure of animal wool. Two elements, i.e. carbon and oxygen were detected in large quantities with SEM-EDS. They are part of wool proteins. All the fibre samples contained aluminum, iron, and calcium originated from mordants.

The mild conditions of dyestuffs extraction enabled to acquire a full fingerprint of the yellow, red, blue, brown, and green dye sources allowing to comprehend how the final colors were obtained. High resolution mass spectrometry facilitated the identification of number of color organic compounds present in the wool fibres extracts, while characteristic fragmentation pathways provided additional information on the structures of the analytes. Four thus-far unknown compounds were found and identified. Two luteolin derivatives (compounds Y12 and Y13) were detected in yellow fibres. Derivative of laccaic acid C - "xantholaccaic acid C" (compound R1) and derivative of laccaic acid A (compound R4), enriched by one hydroxyl group within anthraquinone skeleton were recognized in red fibre extracts. Weld was identified as the dye source in yellow thread samples. Yellow-orange hue of the F4 sample originates from coloration with young fustic and weld. Although analysis of F4 fibre extract did not reveal presence of the brazilein and hematein but identification of "type C" marker in the extract confirms the use of brazilwood as the dyeing source as well. Red fibres have been colored with *Kerria lacca* insect (lac dye). The green fibre shades were obtained with indigo and weld in two baths. Brown wool fibre was dyed with weld, indigo and tannin-containing plant material.

With this study it was possible to contribute for better understanding of what materials and techniques were used for the carpet production. Knowledge of historical artwork components shall enable appropriate preservation of textile treasures with the original materials and methods.

Supplementary Materials: Supplementary materials are available online.

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Conflicts of Interest: The authors declare no conflict of interest.

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