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Identification of Natural Red Dyes in 15-17th Centuries Ottoman Silk Textiles (Kaftans, Brocades, Velvets and Skullcaps) by HPLC with Diode Array Detection

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The high performance liquid chromatography (HPLC) with diode array detection (HPLC-DAD) method was utilized for the identification of dyestuffs of different insect dyes Ararat kermes, cochineal, Mediterranean kermes, lac and dye plant madder the most important natural red dyes found in historical textiles. Reversed-phase HPLC with diode-array detection has been used for identification of natural dyes in extracts of historical silk art objects, from historical textiles and newly dyed silk and wool fibres. The objects examined originate from 15-17th century Ottoman period and belong to the collection of the Topkapi Palace Museum in Istanbul. Fibres with only a red colour were selected for the analysis. Extraction from silk fibres was carried out with HCl/MeOH/H_2O solution. Dye components were identified in the 13 art objects analyzed. Insect and plant dyes were found.

Key Words: Dyestuff, Cochineal, Lac, Madder, Carminic acid, Kermes.

INTRODUCTION

In this study, fibres that were dyed according to the historical recipes and pure dyestuff standards were used as the reference materials for HPLC analysis. Dyestuffs, dye plants and dye insects were identified in the red dyed fibres of the Ottoman silk kaftans, brocades, velvets and skullcaps. The identification of material in an art object from the cultural heritage has received significant attention, because it is important for the development of appropriate conservation and restoration strategies. Natural dyes have the advantages that their production implies the use of renewable resources, causes minimum environmental pollution and has a low risk factor in relation to human health. Some of natural dyes are used by the pharmaceutical

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industry as a basis for drug products and by the food industry[1]. They have been analyzed, initially by thin layer chromatography (TLC) and more recently by high performance liquid chromatography (HPLC) with diode array detection (DAD)[2-9] and mass spectrometric detection (MS)[10-13] which has been successfully utilized to identify the active colouring ingredients in historic yarns, textiles fibres[14], historic maps[15], printed documents and paintings[16] and icons[17,18]. Historical textiles were dyed with natural dyes which were mainly of plant or insect origin. Dye identification for historical textiles is usually based on comparison with known references. In present study, dye sources used for references are as follows.

Cochineal (*Dactylopius coccus* Costa): The dye insect is one of the most important natural dye used to produce fast reds. This dye was discovered by Mexican dyers in about 1000 BC. Its native country is Mexico. The cochineal insect was brought to Europe by the Spanish in the early 16th century after the discovery of America.

Kermes (*Kermes vermilo* Planchon): In the antique period, kermes dyeing was of great importance in the Near East and Southern Europe. The origins probably go back to the Sumerians, that is, to the 3rd millennium BC. The insect was the source of the most important red dye known to the ancient Mesopotamians.

Ararat kermes (*Porphyrophora hameli* Brand): This insect is native to the area of Mount Ararat in Turkish eastern Anatolia and in Armenia. They grow in the salt marshes on both sides of the river Aras (Araxas), which flows past the north side of Mount Ararat and forms the border between Turkey and Armenia. Armenian sources cited their usage in dyeing silk and for the colouring of miniature paintings in the 5th century AD.

Lac (*Kerria lacca* Kerr): This insect is found in India and Southeast Asian countries such as Thailand, Malaysia, Cambodia, Laos and Indonesia. Around 1500 BC, lac was mentioned in one of the oldest kingdom texts of India *Atharvaveda*. The insect was imported from India to the Near East more than 2000 years ago. After the introduction of the American cochineal, the use of lac in dyeing was decreased.

Madder (*Rubia tinctorum* L.): Madder is one of the most important dye plant used to produce fast reds. It has been used from 3500-4000 BC until now for red dyeing.

Gallnut (*Quercus infectoria* Oliver): The plant was used both for dyeing and mordanting.

EXPERIMENTAL

All reagents were analytical grade, unless stated otherwise. High purity water was purified by passing though a Milli-Q treatment system (Millipore, Bedford, MA, USA) and the HPLC mobile phase was prepared using Milli-Q water. The following standard materials have been used as: carminic acid from Sigma, luteolin from Roth (Karlsruhe, Germany), alizarin from Merck (Darmstadt, Germany) and

kermesic acid, pseudopurpurin and purpurin were synthesized by the University of Jordan. For the identification of dyes on textiles the standards are needed. For standards in our work we used dyed wool and silk standards. Wool or silk reference samples dyed with Ararat cochineal, kermes and American cochineal, lac, *Reseda luteola* (weld) and *Rubia tinctorum* (madder) were used as dyed standards. The standard coloured pieces of wool or silk were prepared in the Laboratory for Natural Dyes, Faculty of Fine Arts, Marmara University.

Dyeing procedure[19]: Mordanting and dyeing of silk fibres was performed as below:

Mordanting: In this experiment, mordanting was done prior to dyeing (called pre-mordanting) to assist the adsorption of the dye and to promote good bonding of the dyestuff and the fibre. The most commonly used mordants such as alum (potassium aluminium sulphate), iron [as iron(II) sulphate] and tin [as tin(II) chloride] were chosen. First the silk were submerged in warm water (about 50 ºC) for 0.5 h to relax the silk fibres, which would make the silk more receptive to mordanting and dyeing. Then the mordanting process was carried out according to the historical mordanting recipes[20].

Dyeing procedures: The dyeing procedures were performed in accordance with the historical dyeing method[20]. In the dye bath, the ratio of the silk to dye extract is 1:25; so that, for example, if 1 g of sample is to be dyed, 25 g of dyestuff is used. The temperature of the dye-bath was then gradually raised to about 80 ºC and was kept at this temperature for about 20 min. The temperature of the dye-bath was then allowed to cool to about 30 ºC; then the dyed silk fabric was squeezed, rinsed thoroughly with water and dried in the open air.

In this study, silk and wool were dyed with three different insect dyes (Ararat kermes = *Porphyrophora hameli*, cochineal = *Dactylopius coccus*) and Mediterranean kermes = *Kermes vermilio*) and three dye plants (madder = *Rubia tinctorum*, weld = *Reseda luteola* and oak = *Quercus infectoria*) as it was used in historical recipes. Dyed silk and wool were extracted with MeOH/HCl/H_2O (1:2:1). A water/acetonitrile gradient containing 0.1 % trifluoro acetic acid mobile-phase HPLC method described by Halpine *et al.*[21] and Karapanagiotis *et al.*[18] has been developed for identification of three insect dyes and applied to dyed silk and wool. The same method was applied to some 15-17th century Ottoman silk textiles. It is seen that there are differences between yarns dyed with Ararat kermes and cochineal using the qualitative analysis method. This method discloses whether Ararat kermes or cochineal were used in the specific historical textiles.

Extraction procedure

Samples were prepared as follows: (i) Newly dyed silk and wool samples[22], which were dyed with the insect dyes and madder (*Kermes vermilio*, *Kerria lacca*, *Dactylopius coccus*, *Porphyrophora hameli*, *Rubia tinctorum*) were prepared in the Laboratory for Natural Dyes, Faculty of Fine Arts, Marmara University. (ii) Dye-

stuffs were extracted directly from insects (*Kermes vermilio*, *Kerria lacca*, *Dactylopius coccus*, *Porphyrophora hameli*) and madder. (iii) Mordanted silk (undyed fibres) were extracted. (iv) Dyestuffs were extracted from historical textile samples.

Dyestuff extraction was done using the previously described method[2-4]. For the extraction of dyestuffs, historical textile samples (0.4-1.0 mg) were hydrolyzed with H_2O:MeOH:37 % HCl (1:1:2; v/v/v; 400 µL) in glass conical tubes for precisely 8 min in a water bath at 100 ºC to extract the organic dyes. After rapid cooling under running cold tap water, the solution was evaporated just to dryness in a water bath at 50-65 ºC under a gentle stream of nitrogen. The dry residue was dissolved in 200 µL of the mixture of MeOH:H_2O (2:1; v/v) and was centrifuged at 2500 rpm for 10 min. Twenty five microliters and/or 50 µL of the supernatant was injected into the HPLC apparatus.

Instrumentation: Chromatographic experiments were performed using a Agilent 1100 series system (Agilent Technologies, Hewlett-Packard, Germany) including a model G1311A gradient delivery pump with a 50 µL loop and Rheodyne valve (7725i sample injector), G1315A diode-array detector is performed by scanning from 191-799 nm with a resolution of 2 nm and chromatographic peaks were monitored at 255, 268, 276, 350 and 491 nm, G1322A vacuum degasser and G1316A thermostatted column compartment and the data station was a Agilent Chemstation. A Nova-Pak C_{18} analytical column (3.9 mm × 150 mm, 4 µm, Part No. WAT 086344, Waters) protected by a guard column filled with the same material, was used. Analytical and guard columns were maintained at 30 ºC. The HPLC gradient elution was performed using an adaptation of the method of Halpine *et al.*[21] and Karapanagiotis *et al.*[18]. Chromatographic separation of the hydrolyzed sample was carried out using a gradient elution program that utilizes two solvents: solvent A: H_2O-0.1 % CF_3COOH and solvent B:CH_3CN-0.1 % CF_3COOH. The flow rate was 0.5 mL/min and following elution program was applied (Table-1).

TABLE-1
PARAMETERS FOR HPLC GRADIENT ELUTION

Time (min)	Flow rate (mL/min)	H_2O-0.1 % CF_3COOH (v/v)	CH_3CN-0.1 % CF_3COOH (v/v)
0	0.5	95	5
1	0.5	95	5
20	0.5	70	30
25	0.5	40	60
28	0.5	40	60
33	0.5	5	95
35	0.5	5	95
40	0.5	95	5

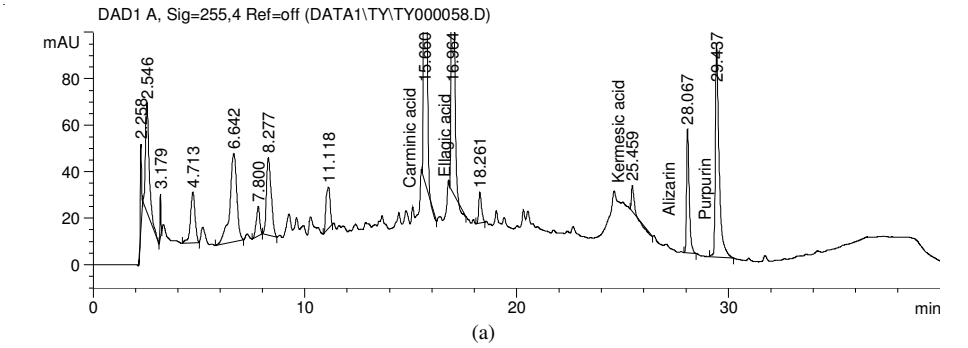
RESULTS AND DISCUSSION

In this study, 13 silk art object (kaftans, brocades, velvets and skullcaps) samples provided by the Topkapi Palace Museum/Istanbul collection were studied (Table-2). Dyestuffs were detected using high-performance liquid chromatography with a diode array detector (Fig. 1). Natural dyes of plant origin-alizarin, purpurin, pseudo purpurin, ellagic acid and tannin derivatives and of insect origin-carminic acid, kermesic acid

TABLE-2
IDENTIFIED COLOURING COMPOUNDS BY HPLC-DAD AND DYE SOURCE OF THE INVESTIGATED ART WORKS FROM TOPKAPI PALACE MUSEUM

Inventory No.	Art object	Sample (colour)	Detected compounds	Identified dye source	Date (century)
13/1515	Silk brocade	Red	Tanin derivatives Ellagic acid Carminic acid Kermesic acid Alizarin Purpurin	*Quercus infectoria* *Dactylopius coccus* *Kermes vermilio* *Rubia tinctorum*	16th
13/1008	Silk brocade	Red	Laccaic acids	*Kerria lacca*	16th-17th
13/1449	Silk velvet	Red	Carminic acid Kermesic acid Ellagic acid	*Dactylopius coccus* *Quercus infectoria*	17th
13/1960	Silk brocade	Red	Carminic acid Ellagic acid	*Dactylopius coccus* *Quercus infectoria*	16th
13/1507	Silk brocade	Red	Carminic acid Ellagic acid	*Dactylopius coccus* *Quercus infectoria*	16th
13/1671	Silk brocade	Red	Carminic acid Kermesic acid Ellagic acid Alizarin Purpurin	*Dactylopius coccus* *Quercus infectoria* *Rubia tinctorum*	17th
13/1918	Silk velvet	Red	Carminic acid Kermesic acid Ellagic acid Pseudopurpurin Alizarin Purpurin	*Dactylopius coccus* *Quercus infectoria* *Rubia tinctorum*	16th
13/1673	Silk brocade	Red	Carminic acid Kermesic acid Ellagic acid Luteolin	*Dactylopius coccus* *Quercus infectoria* *Reseda luteola*	16th
13/1748	Silk brocade	Red	Laccaic acids	*Kerria lacca*	16th
13/1900	Silk velvet	Red	Carminic acid Ellagic acid Alizarin Purpurin	*Dactylopius coccus* *Quercus infectoria* *Rubia tinctorum*	16th

24/1804	Silk skullcap	Red	Ellagic acid Kermesic acid Alizarin Purpurin	*Quercus infectoria* *Kermes vermilio* *Rubia tinctorum*	16th
13/1909	Silk kaftan	Red	Tannin derivatives Carminic acid Kermesic acid Ellagic acid	*Quercus infectoria* *Dactylopius coccus* *Quercus infectoria*	First half of 17th
13/6	Silk velvet kaftan	Red	Carminic acid Kermesic acid Ellagic acid	*Porphyrophora hameli* *Quercus infectoria*	Second half of 15th



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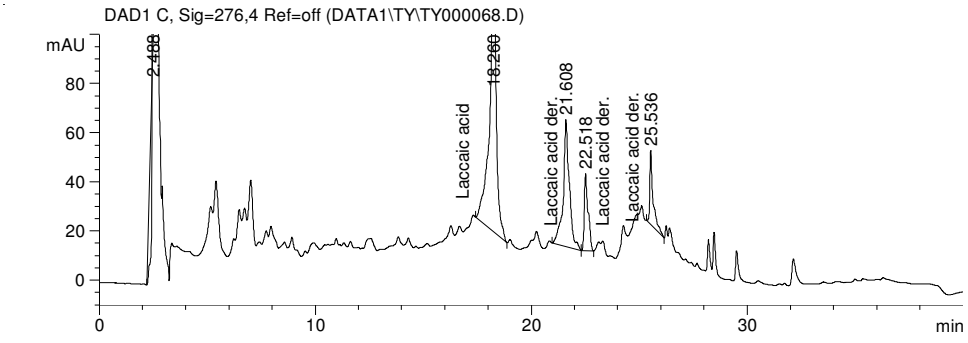

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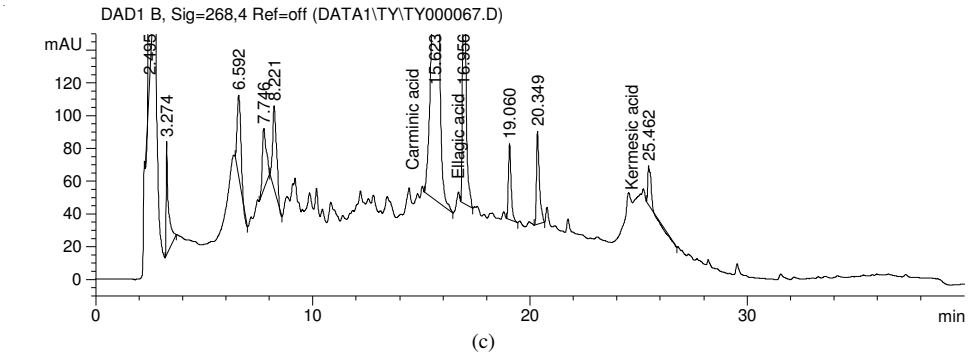

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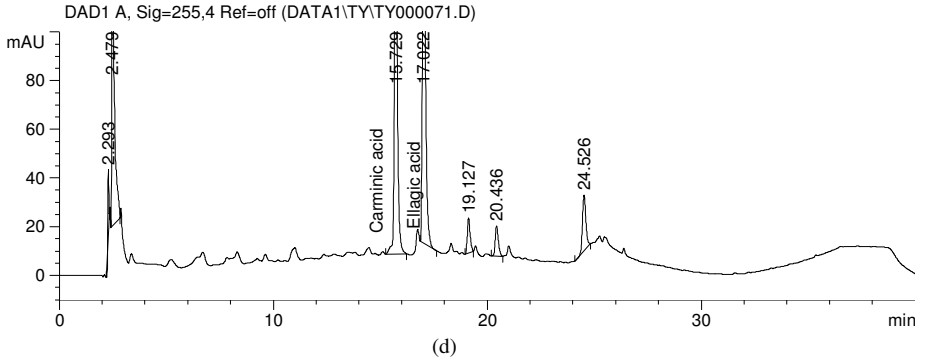
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15.729
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Ellagic acid
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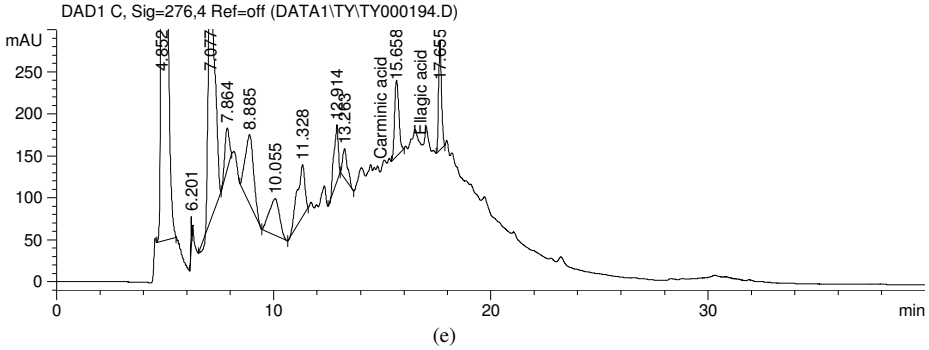
20.436
24.526
min

(d)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7.864
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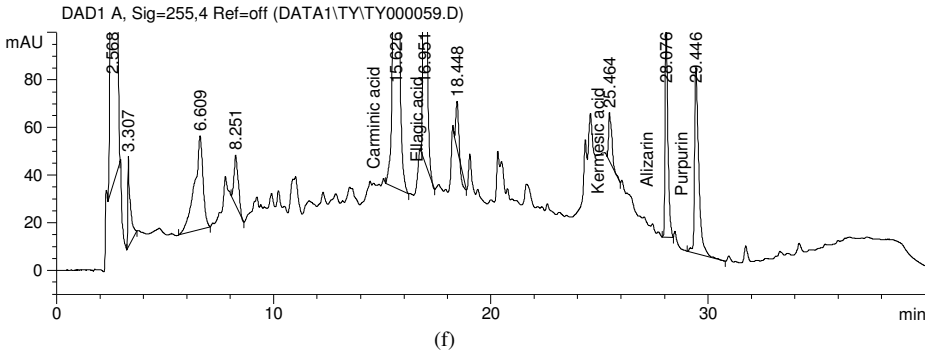
11.328
12.914
13.283
Carminic acid
15.658
Ellagic acid
17.655
min

(e)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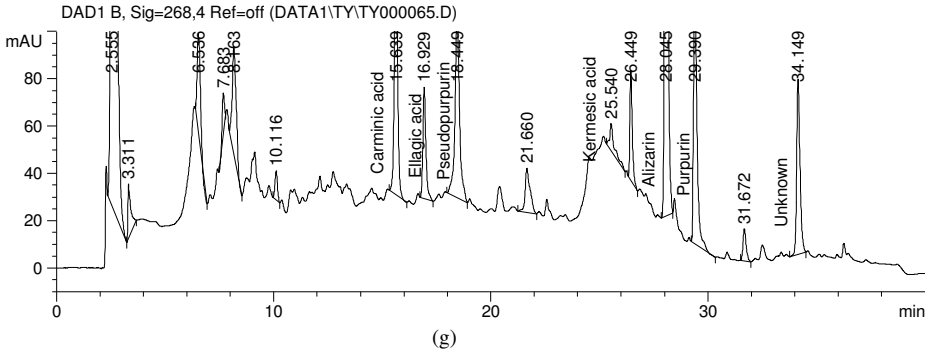
3.307
6.609
8.251
Carminic acid
15.626
Ellagic acid
16.951
18.448
Kermesic acid
25.464
Alizarin
28.076
Purpurin
29.446
min

(f)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6.506
7.683
8.463
10.116
Carminic acid
15.609
Ellagic acid
16.929
Pseudopurpurin
18.449
21.660
Kermesic acid
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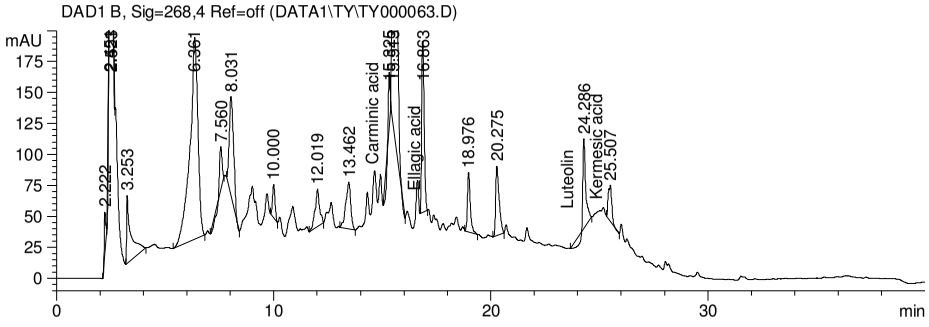
26.449
Alizarin
28.045
Purpurin
29.990
31.672
Unknown
34.149
min

(g)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Ellagic acid
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Luteolin
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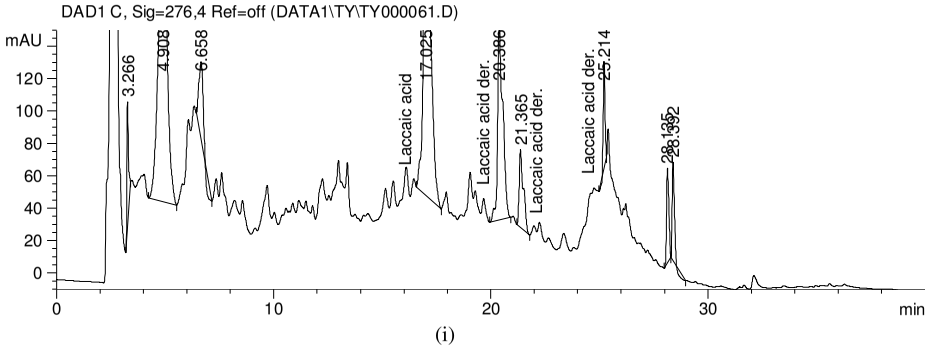
Kermesic acid
25.507
min

(h)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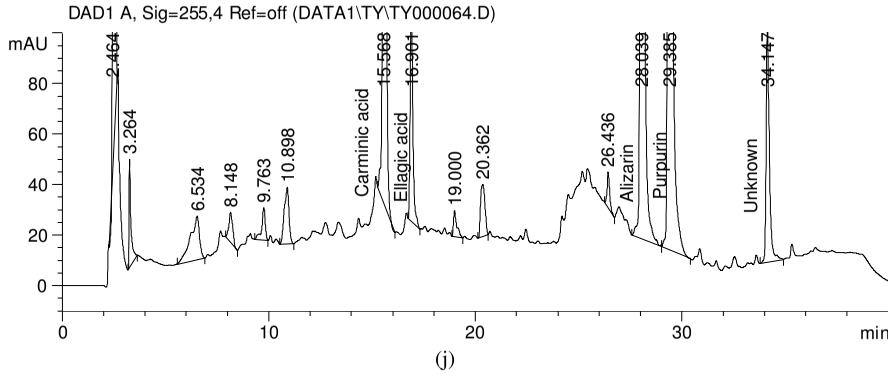
Laccaic acid der.
Laccaic acid der.
21.365
Laccaic acid der.
25.214
min

(i)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Ellagic acid
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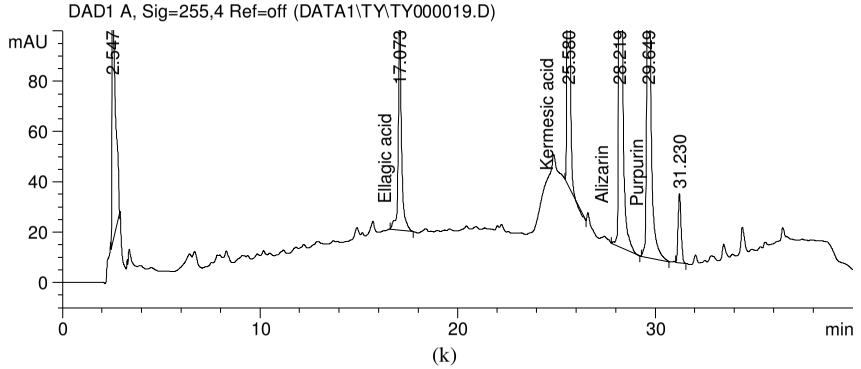
20.362
26.436
Alizarin
Purpurin
Unknown
34.147
min

(j)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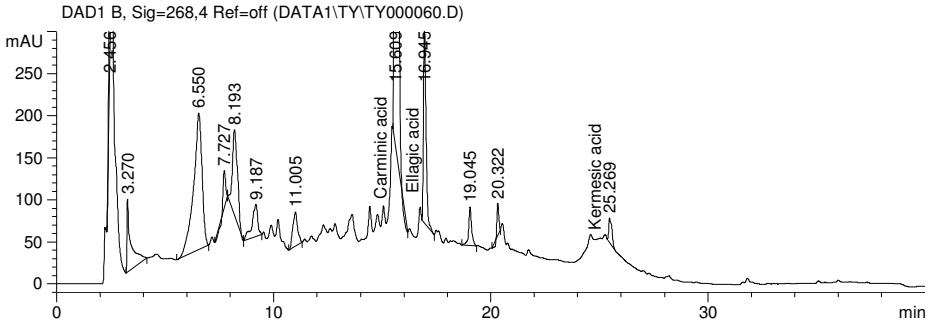
Kermesic acid
Alizarin
Purpurin
31.230
min

(k)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Ellagic acid
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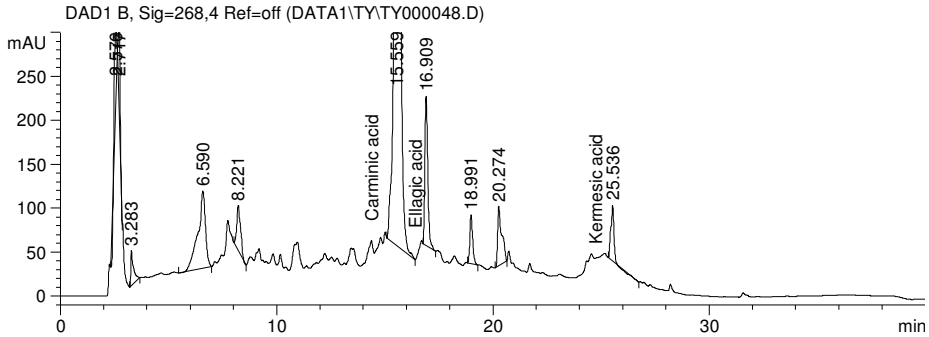
19.045
20.322
Kermesic acid
25.269
min

(l)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Kermesic acid
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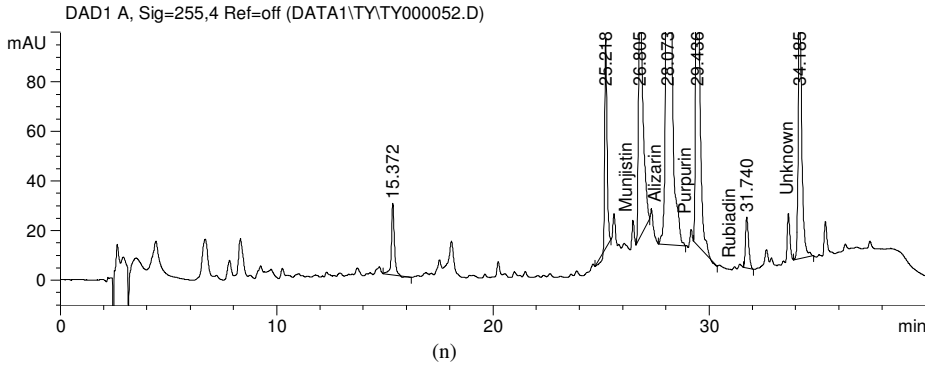
min

(m)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Munjistin
Alizarin
Purpurin
Rubiadin
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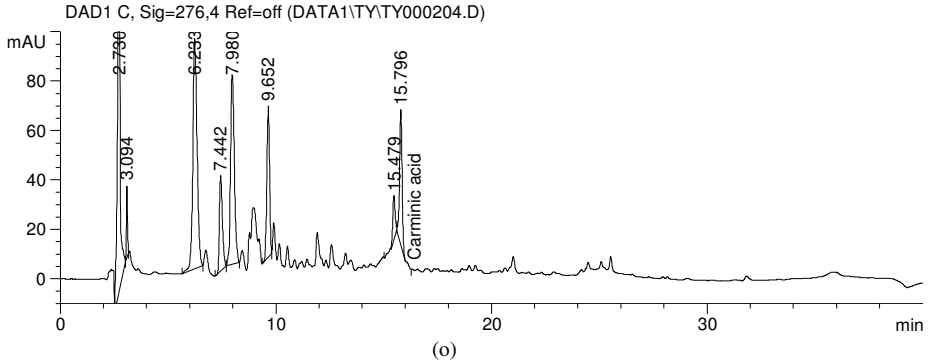
Unknown
min

(n)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15.479
Carminic acid
15.796
min

(o)

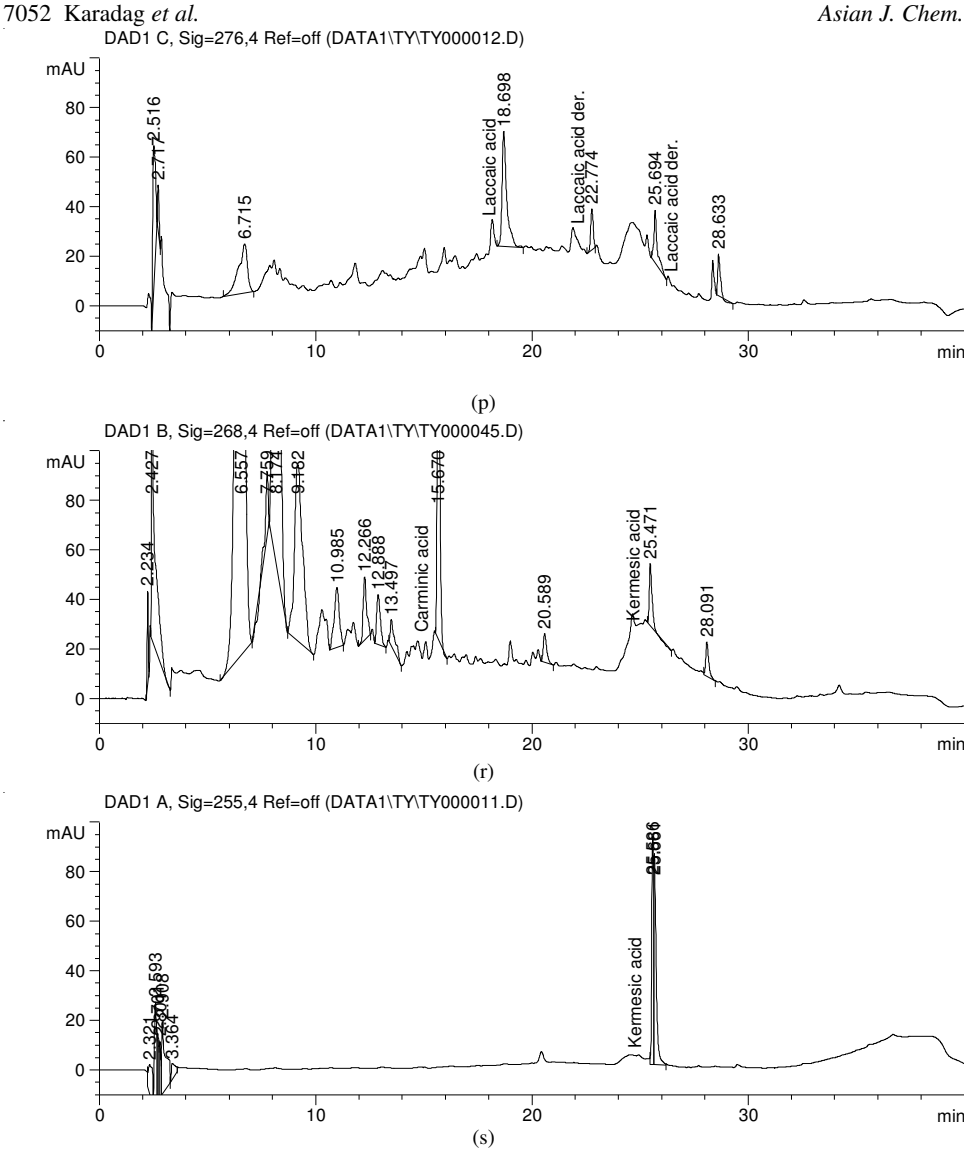
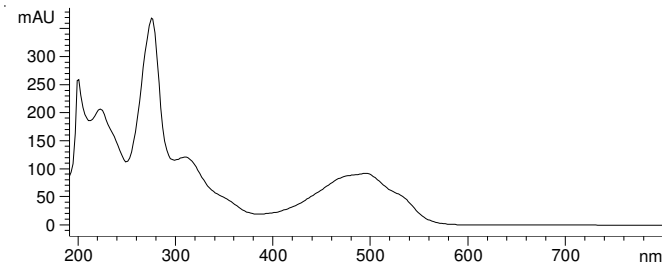


Fig. 1. Chromatograms of historical textiles (according to inventory numbers) and new dyed samples of silk as reference standards (a) 13/1515; (b) 13/1008; (c) 13/1449; (d) 13/1960; (e) 13/1507; (f) 13/1671; (g) 13/1918; (h) 13/1673; (i) 13/1748; (j) 13/1900; (k) 24/1804; (l) 13/1909; (m) 13/6; (n) mader (*Rubia tinctorum*); (o) cochineal and *Quercus infectorius*; (p) *Kerria lacca*; (r) Ararat kermes (*Porphyrophora hameli*); (s) kermes (*Kermes vermilio*)

and laccaic acid-were found. Components were identified as shown in the Table-3. These components of samples were identified based on the absorption spectra acquired with reference standard compounds (Fig. 2). Newly dyed samples of silk or wool for use as reference standards were prepared (Fig. 1). Retention times of silk or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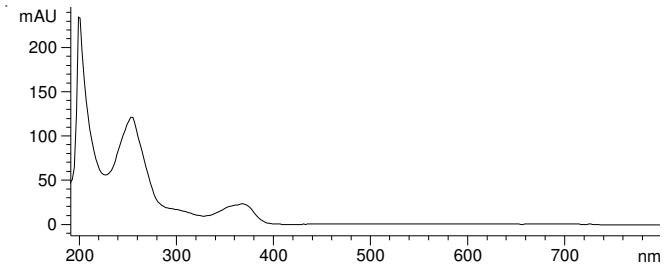
50
0
200
300
400
500
600
700
nm

(A)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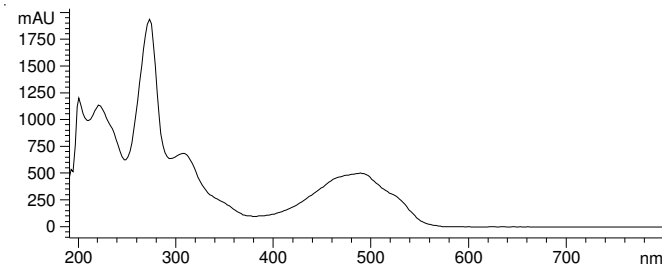
50
0
200
300
400
500
600
700
nm

(B)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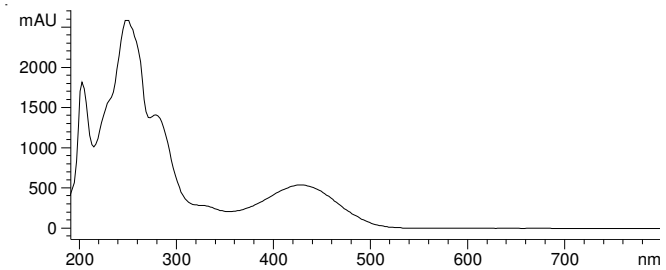
1500
1250
1000
750
500
250
0
200
300
400
500
600
700
nm

(C)


mAU
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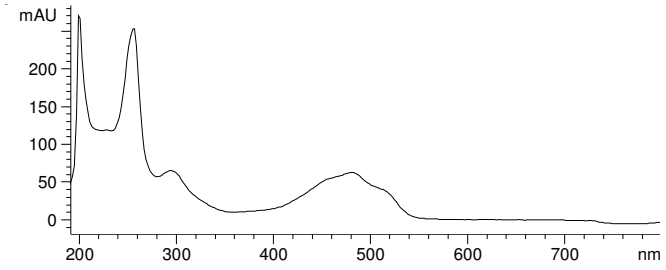
1500
1000
500
0
200
300
400
500
600
700
nm

(D)


mAU
200
150
100
50
0
200
300
400
500
600
700
nm

(E)

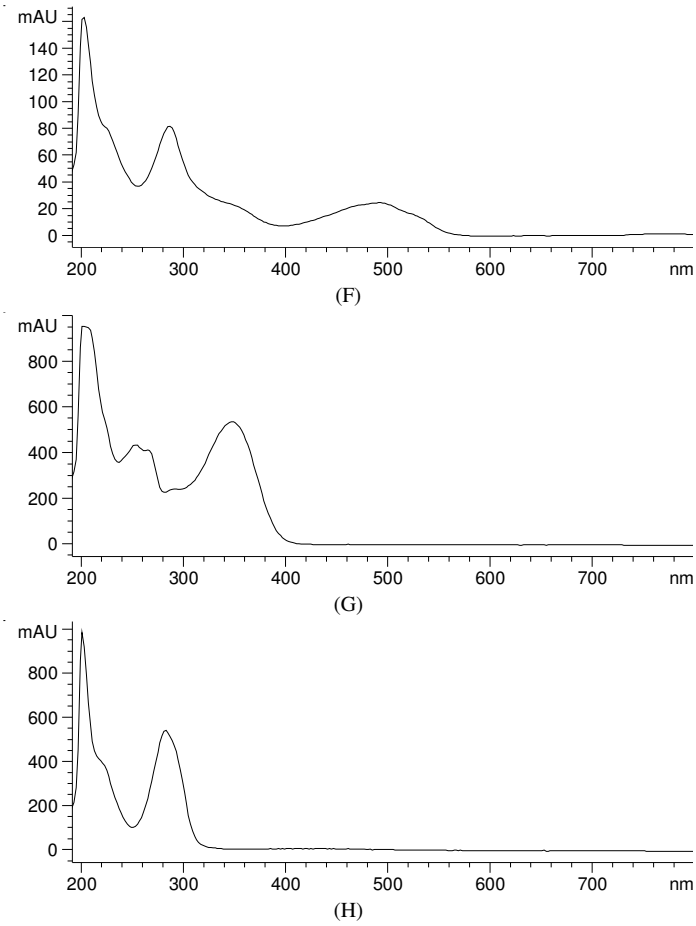


Fig. 2. Absorption spectra in UV-visible regions of the dye standards. (A) Carminic acid; (B) ellagic acid; (C) kermesic acid; (D) alizarin; (E) purpurin; (F) laccaic acid; (G) luteolin; (H) pseudopurpurin

wool standard substances which are dyed using plant and insect dyes are shown in Table-3. One unknown component (Rt 34.149) was identified in inventory No. 13/1918 and 13/1900. This peak was found to belong to *Rubia tinctorum*. *Quercus infectoria* or *Quercus ithaburensis* which contain ellagic acid and tannin derivatives might have been used as a mordant. According to the literature, *Quercus infectoria* has been used as a dyestuff in Ottoman textiles[20]. The sample, inventory No. 13/1673, had been dyed with carminic acid, ellagic acid, luteolin and kermesic acid. Two probabilities are suggested for the red sample: (i) the red sample might be dyed with Mexican cochineal and weld or (ii) a very small yellow sample might be mixed into the red sample. In historical sample, inventory No. 13/6, two compounds were identified including carminic acid and ellagic acid which are the major colouring compounds. In addition, kermesic acid was detected. The approximate

quantity of carminic acid is the same in the Ararat cochineal and Mexico cochineal. The amount of kermesic acid in the Polish kermes is much more than from Ararat kermes and Mexican cochineal. Mexican cochineal was imported from Mexico to Europe and Asia beginning of 16th century. The object was dated as 1453. In all probability, the red sample had been dyed with Ararat cochineal.

TABLE-3
CHROMATOGRAPHIC CHARACTERISTIC OF THE INVESTIGATED ART OBJECTS

Colouring component detected	Rt (min)
Tanin derivatives	4.749; 6.592; 7.077; 7.746; 8.221; 10.055; 11.118
Carminic acid	15.660
Ellagic acid	16.964
Pseudo purpurin	18.078
Laccaic acid	18.698
Luteolin	24.286
Kermesic acid	25.471
Alizarin	28.067
Purpurin	29.437

Conclusion

The development of the combination of instrumental analytical techniques was applied to dye extracts of historical silk art objects (kaftans, brocades, velvets and skullcaps) from the Topkapi Palace Museum collection. In this study, only fibres of red colour were selected for the analysis. High-performance liquid chromatography with diode-array detector has yielded good results in analyses of extracts from historical materials. Natural dyes of plant origin-alizarin, purpurin, pseudo purpurin, ellagic acid, tannin derivatives and of insect origin-carminic acid, kermesic acid, laccaic acid were found.

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