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Natural Pigments from the Gall Oak (*Quercus infectoria* Olivier) Shellac

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In this study, the extract that contains the tannins of gall oak plants (*Quercus infectoria* Olivier) shellac (gallic acid, ellagic acid, tannic acid) and its derivatives were obtained separately. The natural organic pigments were obtained by supplementing aluminum(III) and iron(II) metals into these extracts. 10, 20, 30, 40 and 50 mL solutions of each metal were created by adding to the extract obtained from plants. Qualitative analyses were done with reserved-phase high performance liquid chromatography (RP-HPLC-DAD) of pigments. Gallic acid and ellagic acid constituting pigment with metals in aluminum tannin and iron tannin were determined by comparing their standards. Reversed-phase HPLC with diode-array UV-visible spectroscopic detection has been used in this identification. The extraction of dyestuffs from the natural pigments were carried out HCl/methanol/water (2:1:1; v/v/v) solution. From the results of the HPLC analysis of the gall-oak shellac pigments, it was determined that gallic acid and ellagic acid present in the natural pigments were precipitated by Al(III) and Fe(II).

Key Words: Gall oak, Dyestuff, Pigment, Gallic acid, Ellagic acid, HPLC.

INTRODUCTION

From prehistoric times, humans have made natural dyes by using some plants, animals and inorganic compounds. Humans have left their mark on their environment in the form of painted images, whether in the form of simple hands prints, works of fine art. Until the late 19th century, natural dyestuffs have been used to obtain colour such as purple, violet, red, blue and navy. In these dyes, the mordant method has been used to obtain these colours. Tannin and tannin derivatives have been used in the dyeing of cotton-fibers.

The plant of gall oak (*Quercus infectoria* Olivier) shellac and tanner sumac (*Rhus coriaria*) are rich in tannins[1]. Dyestuffs that are originated from plants and animals of mordant dyestuff are hard to bind onto fiber. In these dyes, the mordant binds onto the fiber. However natural pigments bind to the mordant by chemical bonds and form complexes that are insoluble in water[1].

The natural dye plants are not toxic or carcinogens are not harmful to the environment. These plants only live between 1 to 2 years. Tannins (gallic acid, ellagic acid, tannic acid and their derivatives) which are obtained from the gall oak shellac plants are not toxic or carcinogen[2,3].

Most of the natural pigments are weak organic acids[2]. The gall oak shellacs have been used for dyeing fibers and they have also been used as a pigment since ancient times. Later on, several natural pigments derivatives were proved to exert different biological activities, such as antioxidant, antimicrobial, antifungal, cytotoxic, larvicidal, antiviral, antimutagenic, anti-inflammatory and antiparasite[2-4].

Metal flavonoid or metal anthraquinone complexes formed with metals like aluminum(III) [$KAl(SO_4)_2 \cdot 12H_2O$], iron(II) [$Fe(SO_4) \cdot 7H_2O$] from these dyestuffs are known as natural pigments[5,6]. In alkaline solution, the pigments precipitate as insoluble metal-dyestuff complexes[7]. The composition of the pigments depends not only on the plant species and origin, but also on the procedures used for the extraction from the plants and on the method used for pigment preparation. Moreover, their composition is influenced by ageing processes[8].

The identification of pigments and dyes is one of the most important targets aimed for in the scientific examination of painting, textiles, illuminated manuscripts and other historic and archaeological materials. Thus, several analytical techniques have been used. For example, gas chromatography/mass spectrometry, UV-visible spectrophotometry, thin layer chromatography, high performance liquid chromatography[9], reversed phase liquid chromatography[10-14] and capillary electrophoresis with electrospray mass spectrometric detection, FT-IR spectroscopy and Raman spectroscopy[15]. Of these techniques, high performance liquid chromatography using diode-array detection is ideally suited to the identification of dyes and natural pigments[16].

Colour of a pigment is the result of three combined factors: The spectrum of the light source, the spectral reflectivity of the pigment and the spectral sensitivity of the eye. The CIELAB (1976)-system was introduced to describe colour as a result of these three factors. This system is a three dimensional space, with coordinate axes L*, a* and b*. L* denotes the brightness of the colour (L = 0: black, L* = 100: white), a* represents the green-red axis (a* negative: green, a* positive: red) and b* represents the blue-yellow axis (b* negative: blue, b* positive: yellow). Each pigment colour can be represented as a set of values for L*, a* and b* and consequently as a pigment in this colour space[17].

EXPERIMENTAL

Plant, standard natural dyes and chemicals: All reagent were analytical grade and were treated before using HCl, CH_3CN, $KAl(SO_4)_2·12H_2O$, $FeSO_4.7H_2O$, K_2CO_3 were from Merck (Darmstadt, Germany), $C_6H_2(OH)_3COOH$ (gallic acid), $C_{14}H_6O_8$ (ellagic acid) were from Sigma, pH 4-7 buffers were from Noratex and carboxy methyl cellulose was from Pentalin BNR.

Gall oak (*Quercus infectoria* Olivier) shellac plants were picked from the region Canakkale (West of Turkey) in November and were treated at Laboratory for Natural Dyes, Faculty of Fine Arts, Marmara University, Istanbul. Highly purity water was purified by passing through a Milly-Q treatment system (Millipore, Bedford, MA, USA) and the HPLC mobile phase was prepared using Milli-Q water.

Hanna instruments HI 8314 membrane pH meter, Heraeus D-6450 Hanau Oven, WiseStir MSH-20A Daihan Scientific Co. Stirrer, Shimadzu AEX-200G, Gesellschaft fur labortechnik (GFL) and GretagMacbeth spectroEye spectrophotometer were used.

HPLC equipment: Chromatographic experiments were performed by using an Agilent 1200 series system (Agilent Technologies, Hawlett-Packerd, Germany) including a model G1311A quaternary HPLC pump, G1315A diode-array dedector (chromatograms were obtained by scanning the sample from 191 to 799 nm with a resolution of 2 nm and chromatographic peaks were monitored at 255, 268, 276, 350 and 491 nm), a G1322A vacuum degasser and a G1316A thermostatted column compartment and the data. All of these were analyzed using an Agilent chemstation. A Nova-Pak C_{18} analytical column (3.9 × 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. Chromatographic separation of the hydrolyzed sample was carried out using a gradient elution program that utilizes two solvents: solvent A: H_2O-0.1 % TFA and solvent B: CH_3CN-0.1 % TFA. The flow rate was 0.5 mL/min and following elution program was applied (Table-1).

Extraction: 2 g of dried and powdered gall oak were weighed and transferred into a beaker. Then 150 mL distilled water was added. The mixture of gall oak was heated to 100 ºC with a magnetic mixer. The mixture was held at low temperature for 1 h. Then the mixture was filtered to eliminate impurities and obtain the gall oak extract at 30-35 ºC.

TABLE-1
PARAMETERS OF GRADUENT ELUTION PROGRAM

Time (min)	H_2O + 0.1 %, TFA (%)	CH_3CN + 0.1 %, TFA (%)
0	95	05
1	95	05
20	70	30
25	40	60
28	40	60
33	05	95
35	05	95
40	95	05
45	95	05

Formation of gall oak pigments

Formation of iron-gall oak pigments: 2 g of powdered gall oak pigments was weighed and placed in a beaker. 150 mL distilled water was added in it and mixed well. The mixture was heated up to 100 ºC, then held at low temperature. 10, 20, 30, 40 and 50 mL % 4 $FeSO_4·7H_2O$ solutions and 150 mL of plant extract solution were heated separately to 90 ºC and 60 ºC, respectively. 10 mL from $FeSO_4·7H_2O$ solutions at 90 ºC were added to gall oak pigments solution at 60 ºC. Black coloured iron-gall oak pigments were observed. Afterwards, 0.1 M K_2CO_3 solution was added to neutralize the mixture. The mixture was cooled to room temperature to precipitate the iron gall oak pigment. After setting down, the mixture was filtered and the precipitate was washed with distilled water. The residue was dried on filter paper at 101 ºC for a while. The dried iron gall oak pigment was powdered[14]. The same process was repeated using 20, 30, 40 and 50 mL of iron sulfate solution to each part of 150 mL gall oak pigment extract solution. All of these processes were repeated to obtain iron-gall oak pigments.

HPLC analysis: 2.8 mg of iron-gall oak pigments were hydrolyzed using 37 % HCl-CH_3OH-H_2O (2:1:1; v/v/v/) mixture before chromatographic analysis. The aqueous mixture was evaporated under a Nitrogen flow at 65 ºC. The solid residue was dissolved in CH_3OH-H_2O (2:1; v/v) for analysis. The chromatograms and spectra are given in Figs. 1-3.

Formation of aluminum-gall oak pigments: 2 g of powdered gall oak pigments were weighed and placed in a beaker. 150 mL of distilled water was added to it and mixed well. The mixture was heated up to 100 ºC. Then it was held at low temperature for 1 h. The mixture was filtered at 30-35 ºC to eliminate the impurities. The extract was obtained by this process. The mixture was cooled to room temperature to precipitate the aluminum-gall oak pigment. After settling down,

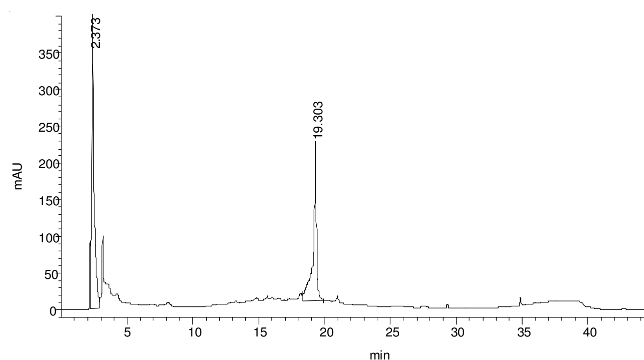

Fig. 1. HPLC chromatogram of the iron-gall oak pigment

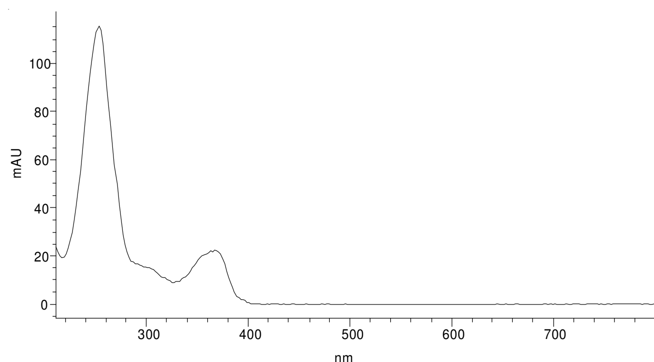

Fig. 2. Photodiode array spectrum of peak of 19.620 min retention time in Fig. 1

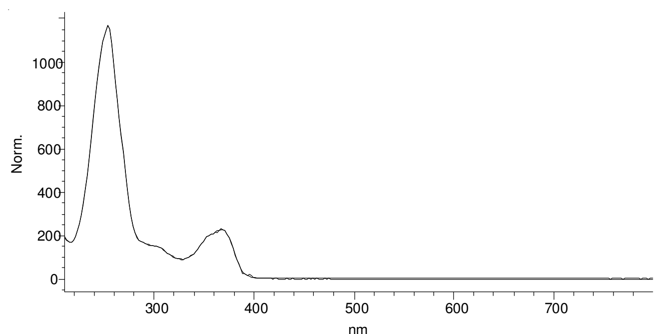

Fig. 3. Photodiode array spectrum of peak of 19.620 min retention time in Fig. 1 and 17.593 min retention time of ellagic acid standard

the mixture was filtered and the precipitate was washed with distilled water. The residue was dried on filter paper at 101 ºC for a while. The dried aluminum-gall oak pigment was powdered[14]. The same process was repeated using 20, 30, 40 and 50 mL of aluminum sulfate salt solution to each part of 150 mL of gall-oak pigments extract solution. All of these processes were repeated to obtain aluminum-gall oak pigments.

HPLC analysis: 2.3 mg aluminum-gall oak pigments were hydrolyzed using 37 % HCl-CH_3OH-H_2O (2:1:1; v/v/v) mixture before chromatographic analysis. The aqueous mixture was evaporated under a nitrogen flow at 65 ºC. The solid residue was dissolved in CH_3OH-H_2O (2:1; v/v) for analysis. The chromatograms and spectra are given in Figs. 4-9.

Colour measurement of gall oak pigments: L*, a* and b* values of pigments were measured with Gretag Macbeth spectro eye spectralphotometer. CIELAB graphs of the pigments were drawn by using of the measured of gall-oak pigments (Figs. 10-13)

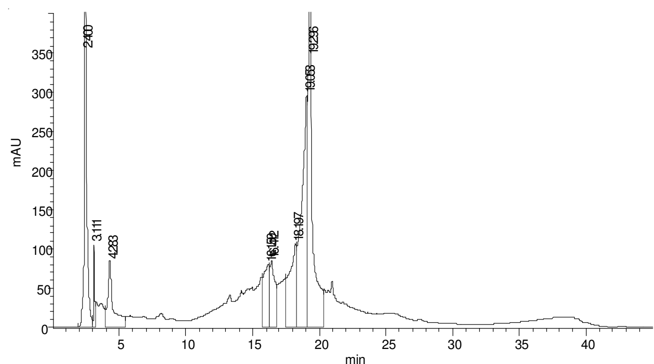

Fig. 4. HPLC chromatogram of the aluminum-gall oak pigment

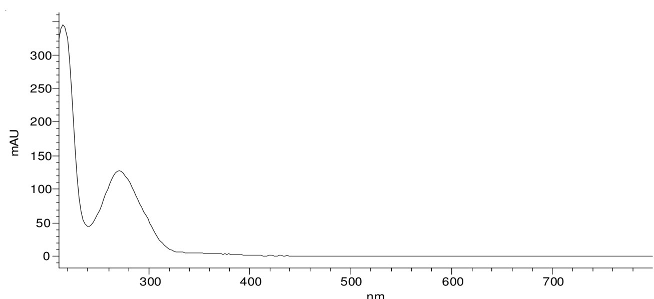

Fig. 5. Photodiode array spectrum of peak of 4.283 min retention time in Fig. 4.

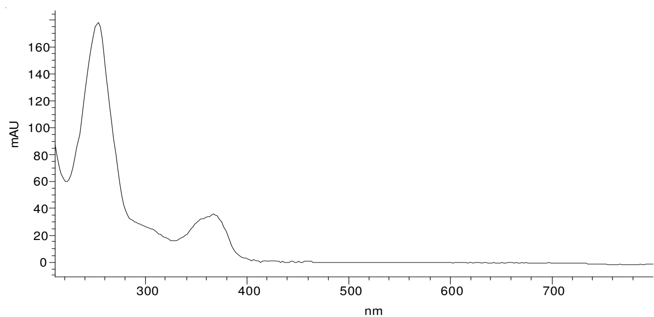

Fig. 6. Photodiode array spectrum of peak of 19.390 min retention time in Fig. 4

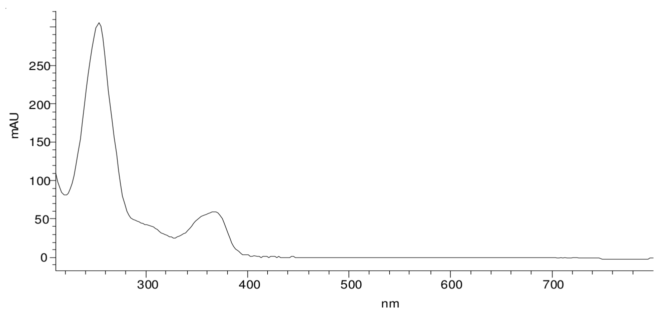

Fig. 7. Photodiode array spectrum of peak of 19.630 min retention time in Fig. 4

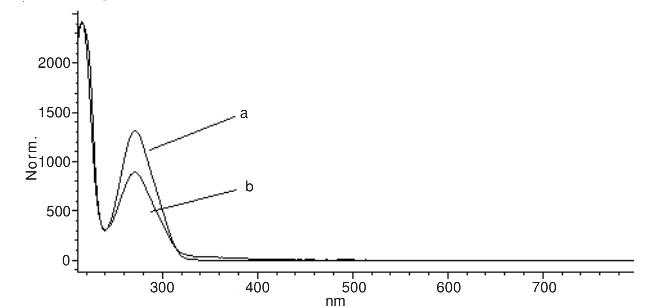

Fig. 8. Photodiode array spectrum of peak of 4.281 min retention time in Fig. 4 (b) 4.602 min retention time of gallic acid standard (a)

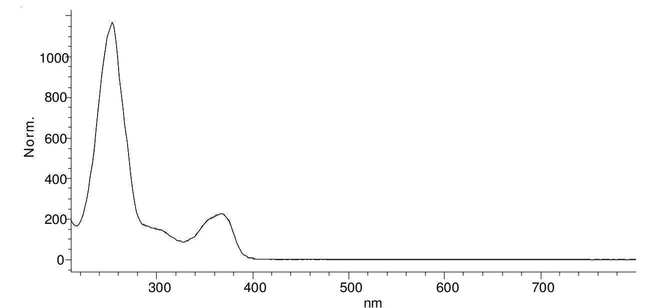

Fig. 9. Photodiode array spectrum of peak of 19.630 min retention time in Fig. 4 and 17.593 min retention time of ellagic acid standard

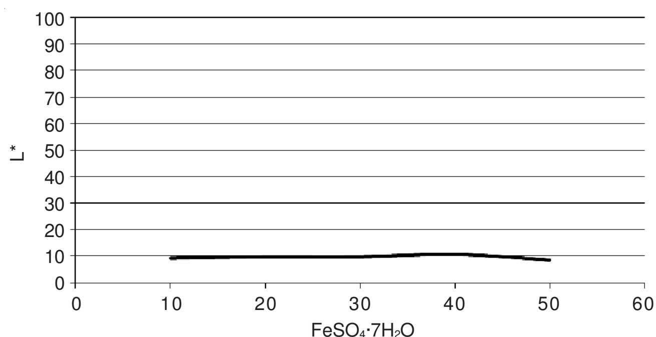

Fig. 10. L* values of the iron-gall oak pigment

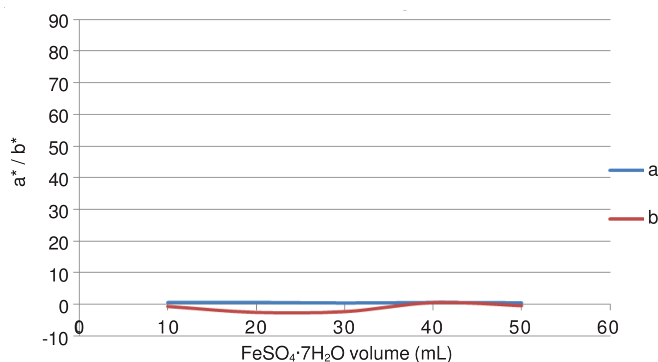

Fig. 11. a* and b* values of the iron-gall oak pigment

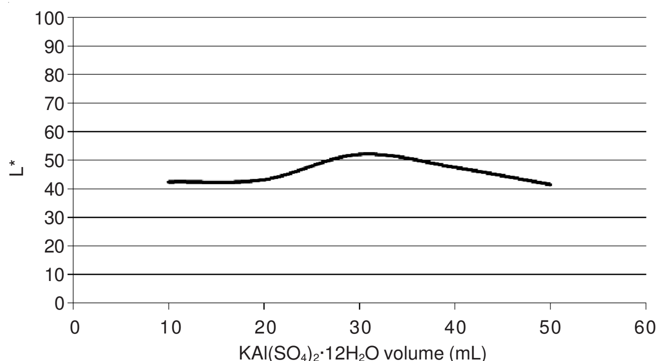

Fig. 12. L* values of the aluminum-gall oak pigment

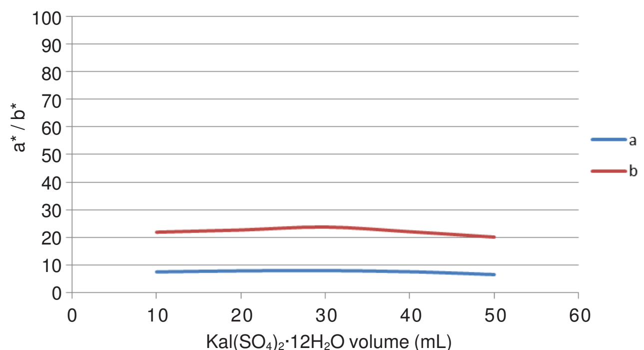

Fig. 13. a* and b* values of the aluminum-gall oak pigment

RESULTS AND DISCUSSION

In the present study, complexes formed with gall-oak (*Quercus infectoria* O.) and iron(II) and aluminum(III) were obtained as natural pigments. The solution of each one metal; 25, 50, 75, 100 and 125 mL were added to the extract obtained from gall-oak and the natural pigments were formed. These gall-oak (*Quercus infectoria* O.) pigments were analyzed quantitatively by a reversed phase high performance liquid chromatography (RP-HPLC). The composition was determined by comparison with standard dyestuffs. HPLC analysis shows that gallic acid and ellagic acid were determined in the acid hydrolyzed gall-oak extract, aluminum-gall oak (pale yellow) and iron-gall oak (black) pigments. The brightness and colour values of iron-gall oak and aluminum-gall oak natural pigments were determined by CIELAB colour space system. The best values for iron-gall oak and aluminum-gall oak natural pigments were observed in samples that were prepared by using 40 and 70 mL of the solution of metal salts, respectively.

Conclusion

In this study, the reaction with Fe(II) and Al(III) of the dyestuffs present in gall oak (*Quercus infectoria* Olivier) has been used to prepare natural gall oak pigments. The result from the HPLC analysis of the acid hydrolyzed gall oak extract is that the iron-gall oak pigment and aluminum-gall oak pigment show that gallic acid and ellagic acid are present. The effect of different volumes of metal solution on the colouring scale of the gall oak pigments were investigated.

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