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ALIZARIN AND PURPURIN CONTENTS OF *RUBIA TINCTORUM* L. ROOTS COLLECTED FROM VARIOUS REGIONS OF TURKEY

TÜRKİYE'NİN DEĞİŞİK YÖRELERİNDEN TOPLANAN *RUBIA TINCTORUM* L. KÖKLERİNDE ALİZARİN VE PURPURİN MİKTARLARI

B.BOZAN[1*], M.KOŞAR[1], C.AKYÜREK[2], K.ERTUĞRUL[2], K.HÜSNÜ CAN BAŞER[1]

[1]Anadolu University, Medicinal and Aromatic Plant and Drug Research Centre (TBAM), 26470 Eskişehir, TURKEY
[2]Selçuk University, Faculty of Science, Department of Biology, Konya, TURKEY

The effect of extraction method on the recovery of alizarin and purpurin from roots of Rubia tinctorum L. was investigated. Alizarin and purpurin contents in Turkish Madder collected from various regions of Anatolia have been determined quantitatively by reversed phase high performance liquid chromatography.

Rubia tinctorum L. köklerindeki alizarin ve purpurin'in geri kazanımı üzerine çeşitli ekstraksiyon yöntemlerinin etkisi incelenmiştir. Anadolu'nun çeşitli yörelerinden toplanmış örneklerde alizarin ve purpurin miktarları ters faz yüksek basınçlı sıvı kromatografisi yöntemi ile belirlenmiştir

Keywords: *Alizarin; Purpurin; Rubia tinctorum; Antraquinone; HPLC*

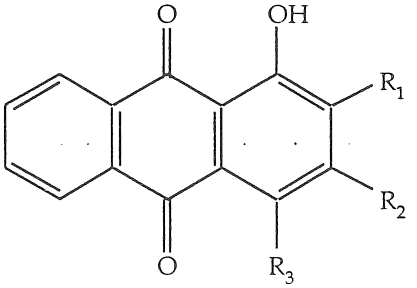
Anahtar kelimeler : *Alizarin; Purpurin; Rubia tinctorum; Antrakinon; YBSK*

Introduction

Before the advent of synthetic dyestuffs, natural sources such as plants and insects were used for coloring textiles. Madder (*Rubia tinctorum* L., Rubiaceae) was formerly of great commercial importance as a dyestuff until mid-19th century.

Rubia tinctorum is a perennial herb flowering from June to August. It is indigenous to the Mediterranean countries of Europe and Turkey and in the past was widely cultivated in France, the Netherlands, Turkey and Central Europe where it often escaped cultivation (1,2). Although madder was cultivated and had great commercial importance for Turkey until the middle of 19th century, it has lost its importance as synthetic dyestuffs have replaced it. Madder was known and used as "Turkey Red" in that period. *Rubia tinctorum* grows wild in all regions of Anatolia (3-5).

R.tinctorum roots when dried and milled yield a variety of colors - red, pink, brown, orange, black, lilac and purple- depending upon the mordant used (2-4). Dyestuffs of the root are anthraquinone derivatives occurring both in free and glycosidic forms. Main anthraquinones are purpurin, xanthopurpurin, munjistin, pseudopurpurin, rubiadin and alizarin (Fig. 1) which was also the first dyestuff produced synthetically.



	R1	R2	R3
Purpurin	OH	H	OH
Alizarin	OH	H	H

Fig.1. Chemical structures of alizarin and purpurin

In the present study, anthraquinone contents (*e.g.* alizarin, purpurin) in *R. tinctorum* roots collected from various

regions of Anatolia were determined quantitatively by a reversed phase HPLC method.

Materials and Methods

Rubia tinctorum roots were collected from Karaman, Muğla, İnebolu, Çoruh, Erzurum, Sivas, Malatya and Beypazarı regions in Anatolia and authenticated by C.Akyürek and K.Ertuğrul. Roots purchased from the local market were used as reference samples. All solvents (HPLC grade) and chemicals (analytical grade) were obtained from Merck (Darmstadt, Germany). Alizarin and purpurin standard samples were purchased from Sigma.

Table 1. Extraction methods tested for recovery of alizarin and purpurin from *Rubia tinctorum* L. roots

Method 1	Soxhlet extraction with MeOH: H_2O (1: 1) 24 h.
Method 2	Maceration with MeOH: H_2O (1: 1) 24 h, in the dark.
Method 3	Maceration with $CHCl_3$ (saturated with water) 12 h, in the dark.
Method 4	Hydrolysis with MeOH: 3N HCl (1:1) at 70°C, 30 min. Aglycones were extracted with toluene.
Method 5	Hydrolysis with MeOH: H_2O: 25% $FeCl_3$: HCl (conc.) (2: 3: 1: 2) at 70°C, 30 min. Aglycones were extracted with $CHCl_3$

Extraction of antraquinones

The effect of extraction procedures on the recovery of antraquinones was performed using various methods as described in Table 1.

Quantitative determination of alizarin and purpurin by HPLC:

Chromatographic conditions:

The LC system used was Shimadzu LC-6A with a Shimadzu SIL-6A automatic injection system. HPLC experiments were conducted using a Ultracarb ODS-C_{20} (5 µm particle size, 15 cm length, 4.6 mm i.d., Phenomenex,U.S.A.) column with a flow rate of 1.5 ml/min at ambient temperature. Shimadzu SPD-6AV UV- Visible detector set at 415 nm was used together with Shimadzu C- R4A Model Chromatopac integrator. MeOH: H_2O: HCOOH (25: 74: 1, v/v/v) (A) and AcCN: H_2O: HCOOH (69.5: 29.5: 1, v/v/v) (B) solvent systems were used in the gradient elution. The elution profile consisted of isocratic elution (50% B) in 10 min, a linear gradient from 50% B to 80% B in 5 min and 80% to 100% B over the next 5 min, isocratic elution (100%B) for 5 min followed by linear gradient.

Sample preparation:

The drug (0.3 g) was hydrolysed under reflux with MeOH: H_2O: 25% $FeCl_3$: HCl (conc.) (2: 3: 1: 2) at 70°C for 30 min. The aglycones were extracted with $CHCl_3$ (5 ml x 3), $CHCl_3$ was removed *in vacuo* and the residue was dissolved in 25 ml MeOH and chromatographed.

Standart solutions:

Standart stock solutions were prepared by dissolving 10.6 mg alizarin and 13.6 mg purpurin in 50 ml methanol. 0.5, 1, 2, 3 and 4 ml of stock solutions were diluted with methanol in a 5 ml volumetric flask. A 10 µl volume of each solution was injected into the column.

Peak areas of the chromatograms were plotted against concentrations (mg/ml) of the sample injected. Results were expressed as the average of three injections.

Results and Discussion

Anthraquinones occur in the root of *R. tinctorum* both in free form and as glycosides of O-type. In the present study, several extraction techniques were tested and the hydrolyses were performed in acidic medium. Results obtained from the study are shown in Table 2. Highest yield for alizarin and purpurin were obtained by the method 5 which involved acid hydrolysis procedure.

Table 2. The effect of extraction techniques on the recovery of alizarin and purpurin in *R.tinctorum*

Extraction methods	Alizarin (%)	Purpurin (%)
Method 1	0.40	0.06
Method 2	0.22	0.01
Method 3	0.14	0.02
Method 4	0.40	0.70
Method 5	0.67	0.40

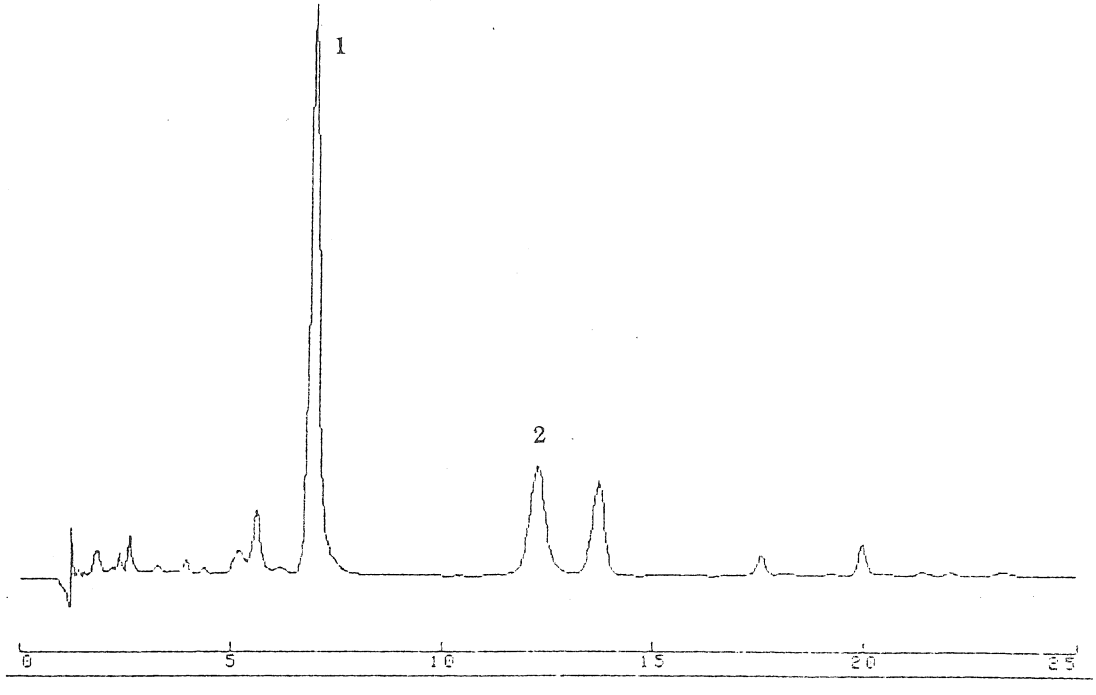


Fig. 2. High performance liquid chromatogram of anthraquinones in *Rubia tinctorum* L. (1-Alizarin 2-Purpurin)

Although, in method 4 and method 5, alizarin and purpurin contents were similar, in method 4 purpurin content was slightly higher, presumably due to the decarboxylation of pseudopurpurin (7). Highest alizarin content was obtained by method 5 while purpurin content was reasonable. Therefore, method 5 was found to be the most suitable among the tested extraction methods for the extracti on of the antraquinones.

Alizarin and purpurin were well separated on an Ultracarb ODS-20 column and eluted within 15 min. Liquid chromatogram of the extract is shown in Fig. 2.

Table 3. The alizarin and purpurin contents in *R.tinctorum* samples collected from various regions

Region	Alizarin (%)	Purpurin (%)
Local market in Istanbul	0.67	0.40
Karaman	1.23	0.80
Muğla	0.61	0.66
Inebolu	0.62	0.61
Çoruh	0.28	0.55
Erzurum	0.82	0.81
Sivas	0.39	0.70
Malatya	0.65	0.48
Beypazarı	0.16	0.26

Peaks with retention times of 7 min and 12 min belonged to alizarin and purpurin, respectively. The peak area of each compound was found to be linear in the range of measured concentrations. The regression equation for alizarin was $y=3139828.6\ x + 1926.5$ ($r= 0.999$) and for purpurin was $y= 1256885.3\ x + 3.3$ ($r= 0.999$), where y is the integration unit and x is the weight in mg per ml of alizarin and purpurin.

Results of the alizarin and purpurin contents in *R.tinctorum* samples were given in Table 3. Highest amount of anthraquinones (alizarin + purpurin) was found in the *R.tinctorum* sample obtained from Karaman region (2.03%).

References

1. Albert, F.: Economic Botany (A Text Book of Useful Plants and Plant Product.), 2nd Ed., Mc Graw-Hill Book Company, p. 130 London 1952
2. Grieve, M.: A Modern Herbal, Penguin Book Ltd., p. 504 England 1982
3. Enez, N.: Doğal Boyamacılık, Mar. Ün. Yay. No: 449 pp. 10-15 İstanbul 1987
4. Dölen, E.: Tekstil Tarihi- Dünyada ve Türkiye'de Tekstil Teknolojisinin ve Sanayinin Tarihsel Gelişimi. Mar. Ün. Tek. Eğt. Fak. Yay. No: 92/1 pp. 472,479 İstanbul 1992
5. Baytop, T.: Türkiye'de Bitkiler ile Tedavi. İst. Ün. Yay. No: 3255 p. 306 İstanbul 1984
6. Doğanca, S., Uygur, A.: Rubia davisiana Ehrend. Antrakinonoları Üzerinde kimyasal Araştırmalar Doğa- Tr. J. Pharm. 1, 17 (1991)
7. Wouters, J.: High Performance Liquid Chromatography of Anthraquinones: Analysis of Plant and Insect Extracts and Dyed Textiles, Studies in Conservation 30, 119 (1985)
8. Berg, W., Hesse, A., Kraft, R., Herrmann, M.: Pharmazie 29 H.7, 478 (1974)

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