

HPLC SEPARATION AND QUANTITATIVE DETERMINATION OF ANTHRAQUINONES IN THE BARKS OF SOME RHAMNUS SPECIES*

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SUMMARY

This research is carried out on the barks of four *Rhamnus* species (*R. libanoticus*, *R. orbiculatus*, *R. pyrellus* and *R. pichleri*). Free and combined anthraquinone extracts were lysed by RP-HPLC. The content and amount of alaternin, emodin, physcion and chrysophanol were determined. Emodin in *R. libanoticus* and *R. pyrellus*, physcion in *R. pichleri* and isophanol in *R. orbiculatus* are major compounds. Alaternin is usually present small amo-

Key Words: *Rhamnus*, Anthraquinones, HPLC.

BAZI RHAMNUS TÜRLERİ ANTRAKİNONLARININ YBSK METODUYLA İNCELENMESİ

ÖZET

Bu çalışma Anadolu'da yetişen 4 *Rhamnus* türünün (*R. libanoticus*, *R. orbiculatus*, *R. pyrellus* ve *R. pichleri*) kabukları üzerinde yapılmıştır. Bağlı ve serbest antrakinon aglikon-taşıyan ekstratlar, ters faz YBSK metoduyla incelenmiş ve kabuklarda bulunan alaternin, emodin, fisikyon ve krizofanol miktarları tayin edilmiştir. *R. libanoticus* ve *R. pyrellus*'ta emodin, *R. pichleri*'de fisikyon ve *R. orbiculatus*'ta krizofanol miktarı yüksek bulunmuştur. Alaternin ise genelde düşük miktarlarda tespit edilmiştir.

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INTRODUCTION

Anthraquinones and anthraquinone containing drugs are important in therapy and have been using as laxatives . Turkey is rich for *Rhamnus* species. 21 *Rhamnus* species which 6 of them are endemic were recorded from Turkey (1). Many researches have been done on the isolation and structure elucidation of the anthraquinones of Turkish *Rhamnus* species (2-5). However there are few studies that carried out by the method of High Pressure liquid Chromatography (HPLC) (6, 7).

In this research the barks of four *Rhamnus* species (*R. libanoticus*, *R. orbiculatus*, *R. pyrellus* and *R. pichleri*) were studied by RP-HPLC. Free and combined anthraquinone containing extracts were prepared from the barks of each *Rhamnus* species and the content of alaternin, emodin, chrysophanol and physcion determined.

MATERIALS AND METHODS

The barks of the studied species were collected mainly from South and East Anatolia between the years 1980-1983. The collection place and date are given in Table 1. Voucher specimens are deposited at "Ankara Üniversitesi Eczacılık Fakültesi Herbaryumu-AEF".

Table 1. The collection place and date of *Rhamnus* species.

<i>Rhamnus</i> species	Collection place and date
<i>R. libanoticus</i> Boiss.	Konya; Ermenek to Karaman, Yellibel Mountain, 2000 m, July 20, 1983.
<i>R. orbiculatus</i> Bornm.	Hakkari, Cilo Mountain, Dize stream, 1500 m, October 1, 1982.
<i>R. pyrellus</i> O.Schwarz	Antalya, Akseki, Murtıçı, 1600 m, September 1, 1980.
<i>R. pichleri</i> Schneider & Bornm.	Antalya to Burdur, Kızılkaya cross, 750 m, September 2, 1980.

HPLC was performed on a Waters M-6000 chromatograph equipped with a pump M-6000A, M-440 UV absorbance detector and a recorder (Houston omniscrite). Separations were carried out on a Waters Nova-pak C18 co-

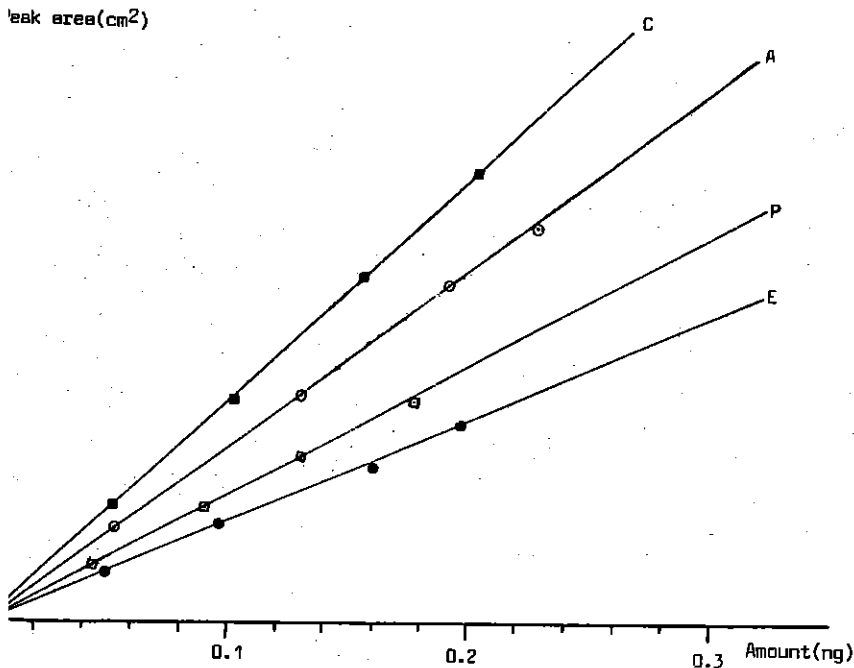
an (4 μ , 15 cmx 3.9 mm ID). Methanol-water (80:20) mixture at a flow rate 1l/min was used. Chart speed, detector sensitivity and column pressure re 2 cm/min, 0.05 AUFS and 3400 psi respectively. Detection was performed at 254 nm.

All solvents were HPLC grade (Merck) and filtered (0.5 μ m fluoropore) a millipore filtering apparatus and degassed prior to use in an ultrasonic water bath for 15 minutes.

The preparation of free and combined anthraquinone containing extracts were carried out by the same procedure given in the previous paper (6). Free filtered (fluoropore 0.5 μ m Millipore) free anthraquinone containing extracts were injected (5 μ l) directly to the column. Combined anthraquinone containing extracts and only free anthraquinone extract of *R. littoralis* were first diluted one to ten, then injected to the column (5

Quantification

The external standard method was used for quantitative determination. Calibration curves were obtained from standard solutions containing anthraquinone aglycones (alaternin, emodin, chrysophanol and physcion) concentration between 10 to 40 μ g/ml. For all substances a linear relationship between peak area and the concentration was observed (Fig 1). All reference standards were isolated from *Rhamnus* species and the purity was checked by HPLC and TLC.



The calibration Curves of Alaternin (A), Emodin (B), Physcion (P) and Chrysophanol (C)

RUSULTS AND DISCUSSION

Reversed phase high pressure liquid chromatography (RP-HPLC) were employed to separate and to determine free and combined anthraquinone aglycones in the barks of four *Rhamnus* species (Fig 3-10). All anthraquinones gave linear detector response when 0.05 μg to 0.2 μg quantities were injected. Methanol: Water (80-20) mixture gave good separation and all anthraquinones were eluted within six minutes (Fig. 2). The results are given in Table 2.

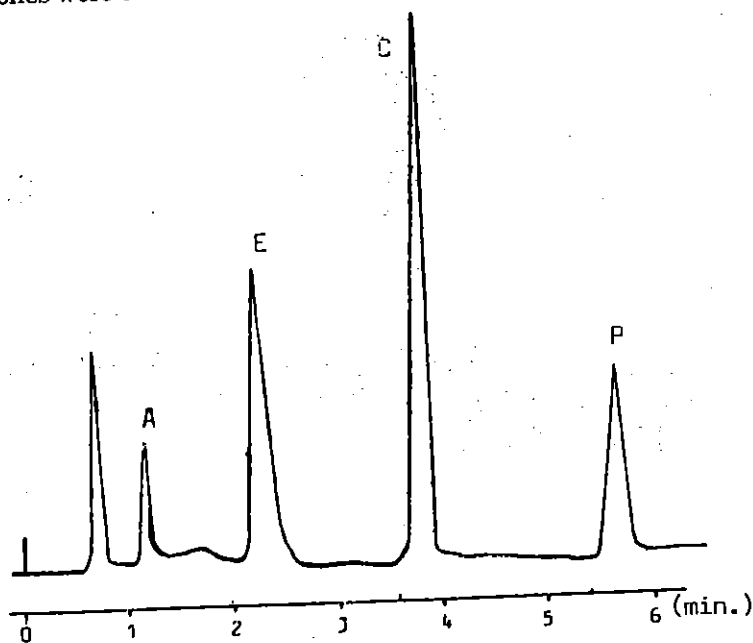


Fig. 2 HPLC Trace of Main Anthraquinones Alaternin (A), Emodin (E), Physcion (P) and Chrysophanol (C)

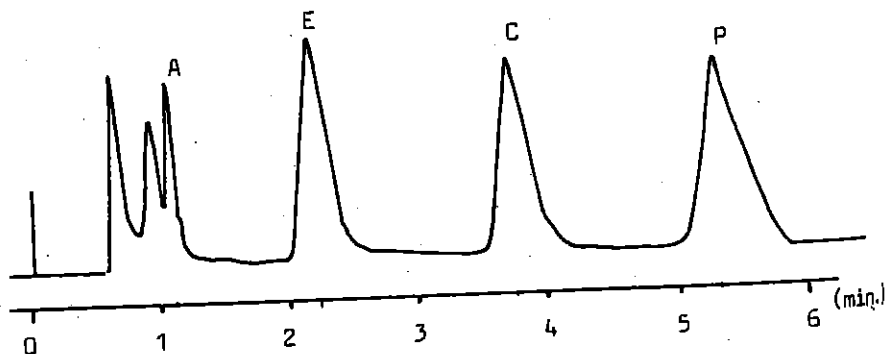
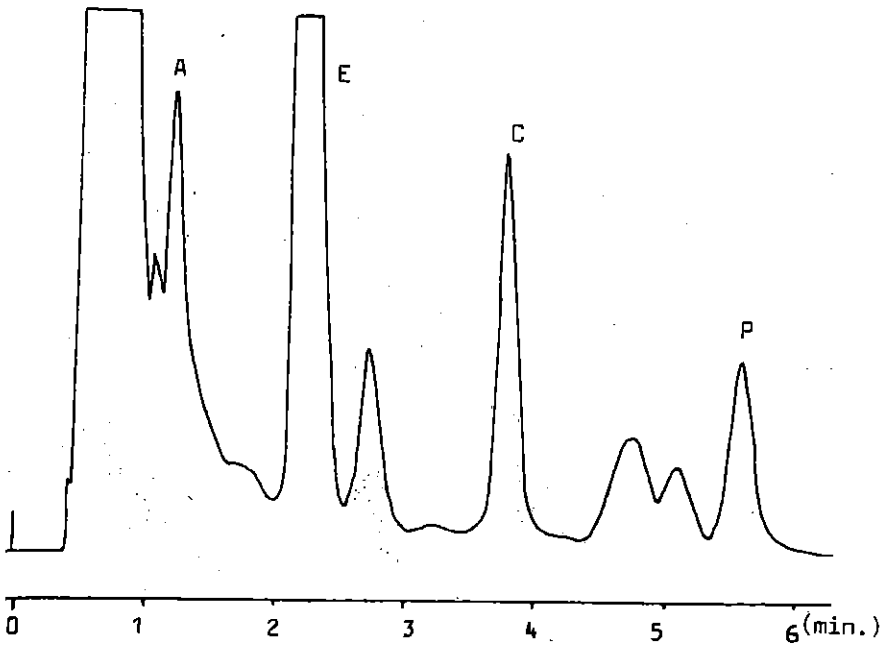
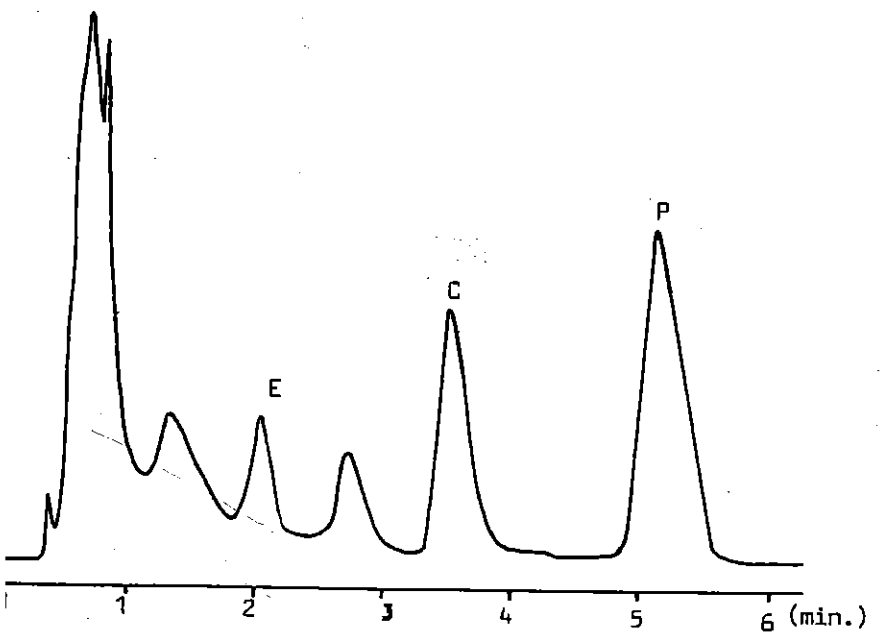


Fig. 3 HPLC Trace of Hexane Extract of *R. libanoticus*.



4 HPLC Trace of ethanolic Extract of *R. libanoticus*



5 HPLC Trace of Hexane Extract of *R. orbiculatus*

Fig. 6 HPLC Trace of Methanolic Extract of *R. orbiculatus*

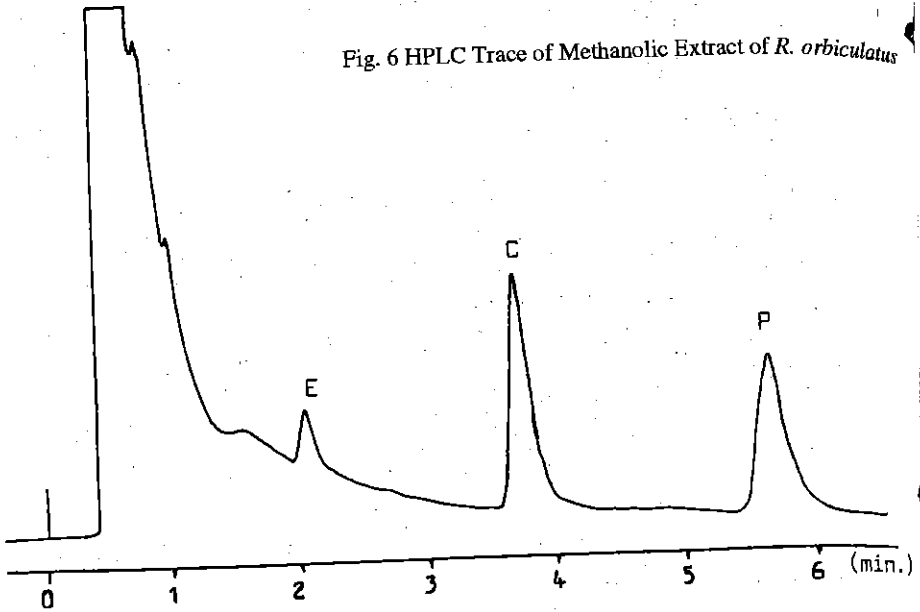
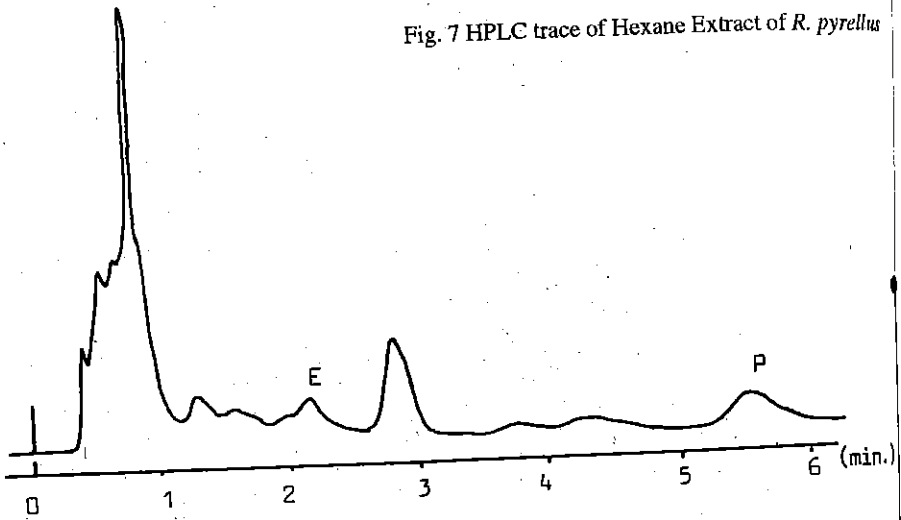


Fig. 7 HPLC trace of Hexane Extract of *R. pyrellus*



Since HPLC is more sensitive method than TLC, combined physcion, free and combined alaternin in *R. libanoticus*; emodin in *R. orbiculatus* and *R. pichleri*; were detected which are not reported in the previous paper (8). The presence of alaternin in *R. pichleri* is not able to corrected due to the large peak at $R_t = 1$ (Fig 9, 10). Among the studied species *R. libanoticus* and *R. pichleri* seem to be rich for anthraquinones. Emodin in *R. libanoticus* and *R. pyrellus*; physcion in *R. pichleri* and chrysophanol in *R. orbiculatus* are the major anthraquinones. Alaternin is usually present in trace amount (Table 2).

Table 2. Anthraquinone content (g/100 g) in the bark of *Rhamnus* species

Species	Emodin		Physcion		Chrysophanol		Alaternin		
	Free	Comb. Total	Free	Comb. Total	Free	Comb. Total	Free	Comb. Total	
<i>R. libanoticus</i>	0.014	0.11	0.014	0.018	0.008	0.016	0.0024	0.0012	0.0036
<i>R. orbiculatus</i>	0.0005	0.0004	0.0003	0.00014	0.001	0.01	-	0.0004	0.0004
<i>R. pyrellus</i>	0.0004	0.0056	0.0001	0.004	-	0.0002	-	0.0002	0.0002
<i>R. pichleri</i>	0.001	0.004	0.01	0.018	0.002	0.01	-	-	-

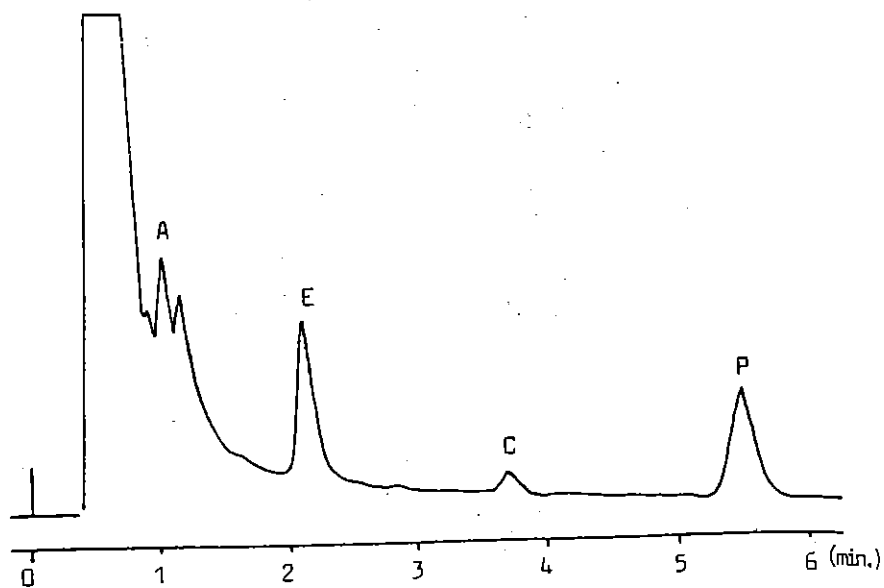


Fig. 8 HPLC Trace of Methalonic Extract of *R. pyrellus*

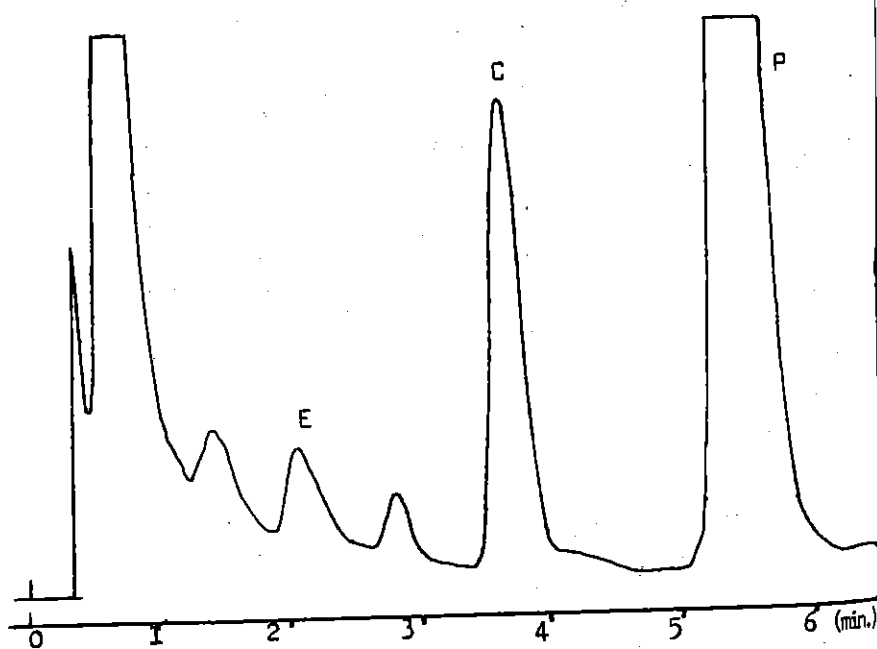


Fig. 9 HPLC Trace of Hexane Extract of *R. pichleri*.

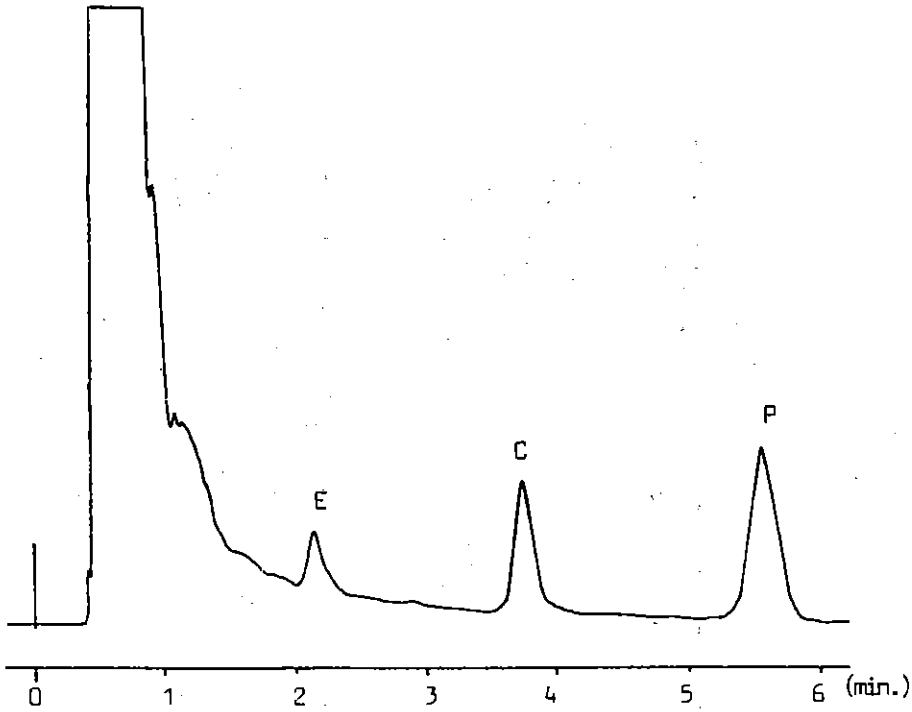


Fig. 10 HPLC Trace of Methanolic Extract of *R. pichleri*.

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