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## High Performance Liquid Chromatographic Method for Quantitative Determination of Emodin in *Rumex Cyprius* Marb, Spectrophotometric Studies

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### Abstract

A quantitative method is used for determination of emodin in *Rumex cyprius* Marb., using high performance liquid chromatography. The plant parts: roots, stems, and leaves, were soaked in 95% methanol solution for 48 hours. Plant parts and standard emodin were eluted from C18 column with a mobile phase consisting of methanol: water (80%:20% volume/volume) at a flow rate of (2.0 milliliter / minute). The effluent was monitored with ultraviolet detector set at 258 nm. Standard curve for emodin was linear in the range of concentration 1.35- 45.0 ppm. The method was applied for determination of emodin in roots, stems and leaves of *R. cyprius* Marb. The results showed that the concentration of emodin in roots, stems, and leaves were 0.16%, 0.04%, and 0.30% respectively in dried plant sample. On the other hand the results obtained showed that emodin exhibits two absorption maxima at 410nm and at 510nm. The absorption peak at 410nm was found to decrease gradually by increasing pH, while the absorption peak at 510nm was found to increase gradually by increasing the pH.

**Keywords:** *Rumex cyprius* Marb.; emodin; HPLC.

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## 1. Introduction

Emodin (1, 3, 8-trihydroxy-6-methylanthraquinone) (Figure1); and its derivatives a naturally occurring anthraquinones are found in different various families such as Rhamnaceae, Liliaceae Caesalpiniaceae and Polygonaceae. *Rumex japonicus* Houtt., a rich source of anthraquinones, has been traditionally used for the treatment of heat phlegm, jaundice, constipation, scabies, and uterine hemorrhage in East Asian countries such as China, Korea, and Japan [1]. Emodin isolated from Polygonaceae such as *Rumex cyprius* Marb., is an important class of natural compounds with widespread distribution and a wide range of biological activities such as hepatocytes and cholangiocytes [2], anti-cancer properties [3], anti-inflammatory activity [4], anti-angiogenic activity [5], and inhibitory activities on protein glycation and aldose reductase [6]. Every year a large number of anthraquinones, having varied substitution patterns are isolated from nature [7, 8].

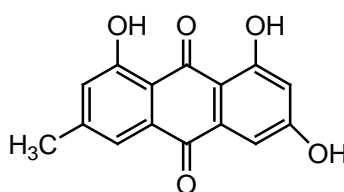


Figure1: Structure of emodin

*R. cyprius* is considered as a medicinal plant in Palestine and is used in folk medicine in the treatment of human skin diseases [9]. Several active chemical constituents were found in various species of *Rumex* including: chrysophanol, physcion and emodin [10, 11] which are naturally occurring anthraquinones, have antimycotic activity against dematophytic fungi such as *Trichophyton rubrum* and *Microsporum canis* [12-14]. A few methods had been used for the determination of anthraquinones such as chrysophanol, physcion and emodin from medicinal plants. There have been some reports on separation of quinines such as High speed counter-current chromatography [15] and using modified mobile phase [3]. Several scientific studies of its biological activity have been performed. More recently, investigations are stressed towards spectral methods. The purpose of this article is to describe a simple, rapid, accurate, and reproducible high performance liquid chromatography (HPLC) method for the determination of emodin in roots, stems, and leaves of *Rumex cyprius* Marb., and to study the effect of acidity of emodin solution on the absorption peak .

## 2. Materials and Methods

### 2.1 Reagents and Chemicals

Emodin was purchased from Sigma, (Sigma, Aldrich GmbH, Sternheim, Germany). HPLC-grade water and methanol was used as HPLC eluent, solvents and all other chemicals and reagents were of analytical grade.

### 2.2 Chromatographic Equipment

High performance liquid chromatographic determinations were carried out using a Gilson chromatograph which consists of two pumps models 302 and 303, dynamic mixer model 811, manual sample injector Rheodyne 7125,

HM / Holochrome UV-visible detector with 190 – 600 nm, and Gilson chart recorder model N 1 single pen .Analysis was performed on a Whatman Partisil 50D-3 HPLC pre-packed (250 x 4.0 mm I.D.5 µm C18) steel column protected with an inlet filter and guard column. UV-VIS measurements were recorded on JENWAY 6800 UV-Vis. spectrophotometer.

### **2.3 Standard Solutions**

A stock solution of emodin ( $5.00 \times 10^{-4}$  M) was prepared by dissolving 13.52mg emodin accurately in 100 mL of methanol solution (80% v/v) .This stock solution was diluted to give the working standards.

### **2.4 Spectrophotometric studies**

The absorption spectra of emodin were studied in the wavelength range 300-700 nm and in the pH range 2.00-13.00 Figure 2.

### **2.5 Chromatographic Conditions**

The mobile phase consists of methanol: water with the ratio (80%: 20% v/v). It was degassed daily. The flow rate was 2.0 ml/min. The detector was set at 258nm and the injection volume was 20 µL. All measurements were performed at ambient temperature. The peak sensitivity was 0.02 and the running time was 6 min.

The choice of these conditions was not arbitrary. The absorbance wavelength was the same as the obtained from the spectrophotometric method of analysis. The choice of the mobile phase ratio was selected after different ratios were tried in order to establish the optimum ratio.

The selected ratio of 80%:20% v/v of methanol to water was found to give the most clear peak and retention time data.

### **2.6 Preparation of samples for analysis**

The whole mature plant screened in this study were collected in April-June 2013 from Nablus area and were identified by Prof. M. S. Ali-Shtayeh from the Biodiversity and Environmental Research Center, BERC, Til Village, Nablus. Voucher specimens (BERC-BX-C-0468 ) is deposited in the Herbarium of BERC. Methanolic extracts were prepared from dried plant parts using soxhlet extractor and the solvent removed in vacuum. The residue was dissolved in a minimum amount of chloroform and subjected to column chromatography using silica gel and chloroform: ethyl acetate (3:7 v/v) as eluent. One of the main constituents was obtained as an orange long crystalline substance with  $R_f = 0.51$  and melting point 255-257 °C and <sup>1</sup>H NMR spectra: (CDCl<sub>3</sub>) δ 2.47 (3H, s, 3-Me), 6.67 (1H, d, J= 2.45, H-5), 7.15 (1H, br s, H-2), 7.26 (1H, d, J= 2.45, H-7), 7.56 (1H, br s, H-4), 12.8 and 12.21 (2H, s, 1/8-OH). Data collected from spectroscopic analysis, melting point, ( $R_f$ ) value, and comparing the substance with an authentic emodin sample, showed the compound to be emodin. The plant was divided into three parts: roots, stems, and leaves. 10.0g of roots, 10.0g of stems and 5.0g of leaves each soaked in 100 mL 95% methanol for 72 hours. A 20µL of each sample was used for single HPLC injection. All experiments were carried out in triplicate.

### 3. Results and discussion

#### 3.1 Absorption spectra

The results obtained showed that emodin exhibits two absorption maxima at 410nm and at 510nm (Figure 2). The absorption peak at 410nm was found to decrease gradually by increasing pH, while the absorption peak at 510nm was found to increase gradually by increasing the pH. These observations confirmed that the absorption peak at 410nm was due to the acidic form of emodin, while the absorption peak at 510nm was due to the basic form of emodin.

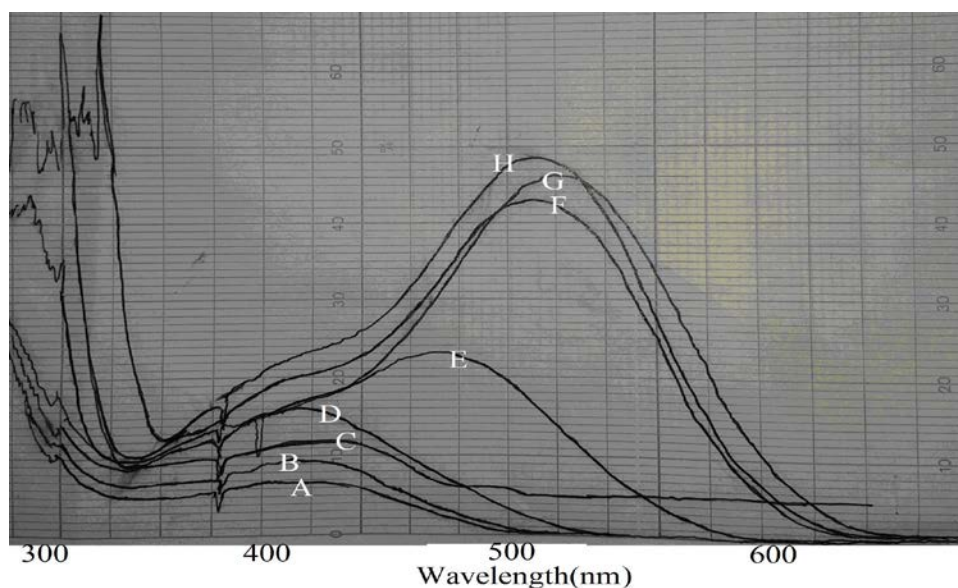


Figure2: Absorption spectra of emodin at various pH values

A- 2.7, B- 4.4, C- 5.3, D- 7.0, E- 7.5 , F- 8.1, G- 10.6, H-12.0

#### 3.2 Stability of the color

The color is attained directly after the addition of the buffer solution and it remains constant for at least 24 hours.

#### 3.3 Calibration Curve

Following the recommended procedure, a linear relationship was obtained between the peak-height and the concentration of emodin ( $Y=0.6897X+0.0445$ ,  $R^2=0.9974$ ) within the range 1.35- 54 ppm Figure 3.

#### 3.4 Applications

The method was applied for the determination of emodin in roots, stems, and leaves of *R. cyprius* Marb. The samples were prepared as described in the general procedure. The results obtained showed that the concentration of emodin in roots, stems, and leaves were 0.16%, 0.04%, and 0.30% respectively in dried plant sample.

Emodin measured by the developed method for various parts of *R. cypricus* Marb, showed that the contents of leaves and fruits are comparatively higher than stems. The proposed method stability is new approach to isolate and formulate emodin content. It will be definitely helpful for the quantification of drug formulations in various plant parts, thus it will be useful for the validation study of plant parts as well as drugs.

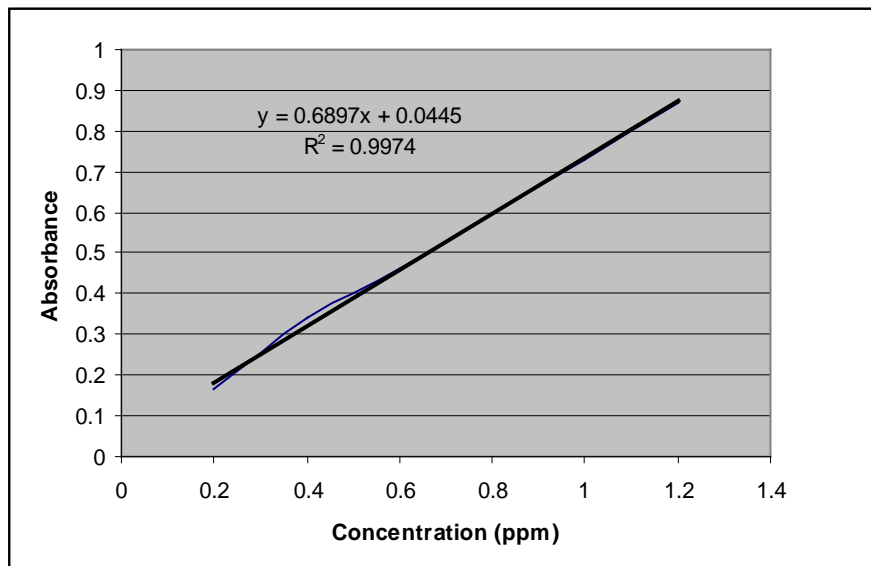


Figure 3: Calibration curve for emodin

#### 4. Conclusion

Emodin, one of the anthraquinones can be determined quantitatively from different parts of medicinal plants such as *Rumex cypricus* Marb. effectively by using HPLC method.

#### Acknowledgments

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#### Declaration of interest

The authors report no conflicts of interest.

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