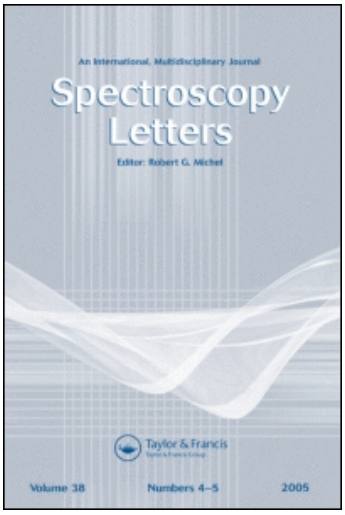

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Emodin, A Naturally Occuring Anthraquinone: Its Isolation and Spectrophotometric Determination in Rumex Cyprius Plant

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EMODIN , A NATURALLY OCCURING ANTHRAQUINONE : ITS ISOLATION AND SPECTROPHOTOMETRIC DETERMINATION IN RUMEX CYPRIUS PLANT

Key Words : Emodin , Spectrophotometric Determination , Isolation , Rumex Cyprius

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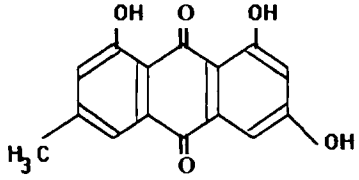
Abstract :

A new method for isolation and specrophotometric determination of emodin is presented. Emodin was isolated by thin layer chromatography (tlc) and column chromatography (cc) techniques , as an orange long crystalline substance. Emodin exhibits two absorption maxima , at 420 and 520 nm . Stability of the color and the effect of pH were studied. Beer's law is obeyed in the range 2 - 30 ppm.

The method is applied to the determination of emodin in roots , stems, and leaves of Rumex cyprius plant.



Emodin 1

Introduction :

Emodin (1 , 3 , 8 - trihydroxy - 6 - mehtylanthraquinone) **1** , a naturally occuring anthraquinone found in older *Rhahmanus frangula* L. in *Ceseara sagraela* also in *Rumex cyprius* and other polygonaceae , has multivarious effects in pharmacology (1 , 2 , 3). Rumex cyprius is a plant used in folk medicine to cure human skin diseases in West Bank (4 , 5). In the present work , two methods have been described for spectrophotometric determination of emodin in roots , stems, and leaves of Rumex cyprius.

Experimental

Chemicals And Reagents

Emodin 0.005 M : The proper weight of emodin was dissolved in 100 ml of ethanol.

Buffer solutions :

The buffer solutions in the pH range 2-13 were prepared from acetic acid , boric acid , phosphoric acid and sodium hydroxide mixtures.

Apparatus :

SP Unicam UV/Vis Spectrometric UV2 was used with aquarts cell (1 x 1 cm). All measurements were performed at room temperature (22°C).

General Procedure

Separation of emodin by column chromatography :

The plant material was collected in spring dried and divided into three parts : roots, stems , and leaves. Ten grams of each part were soaked in 95% ethanol for 7 days. Ethanol was evaporated *in vacuo*. The residue was subjected to column chromatorgraphy using silica gel and chloroform-ethyl acetate [3:7] as eluent. Emodin was obtained in a pure form as an orange long crystalline substance , with an R_f = 0.51 and melting point = 256 - 257°C (6).

Thin layer chromatographic separation of emodin :

The thin layer chromatography method has been used for separation of Rumex cyprius components. Two spots were detected under UV light (362 nm)with R_f values of 0.51 and 0.68.

Upon spraying the dry chromatogram with buffer solution pH 12 , emodin was the only spot which gave a violet colour while the other component did not change in colours.

Spectrophotometric determination of emodin :

A portion of solution containing an amount of emodin in the range 10-150 μg was transferred into 5 - ml volumetric flask. The volume was completed with the buffer solution pH 12 and the absorbance was measured after 2 minutes at 520 nm against buffer solution as a reference.

Preparation of samples for spectrophotometric analysis:

10 g of each part of the plant (roots , stems , and leaves) were soaked in 100 ml 95% ethanol for 7 days. The solutions were filtered and the volumes were adjusted to 100 ml in volumetric flasks.

Results And Discussion

Absorption spectra :

The absorbance of emodin solution which was prepared according to the general procedure has been studied in the wavelength range 350-700nm and in the pH

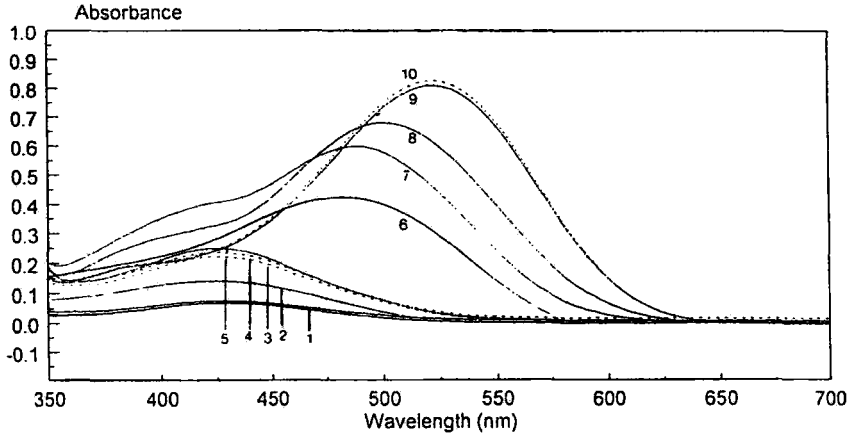


Figure 1 : Absorption spectra of 9.0 X10 $^{-5}$ M emodin at various pH values:

1) pH = 1.0 pH = 2.4 3) pH = 3.5 4) pH = 4.7

5) pH = 5.6 6) pH= 6.3 7) pH= 8.2 8) pH= 10.0 9) pH=12.0

10) pH =14.0

range 1.0 - 14.0. The results obtained showed that emodin exhibits two absorption maxima at 420 nm and 520 nm as shown in Figure 1.

It was found that an increase in the pH will affect an increase in the absorbance at 420 nm up to pH 3.5. This increase was due to the dissociation of the triprotic acidic form of emodin H_3A to H_2A^-. Any further increase in the pH did not affect the absorbance up to pH 5.6 After that, a graduate increase in the absorbance was observed by increasing the pH. This second increase was due to the dissociation of H_2A^- to HA^{2-}. The absorbance at 420 nm reached its maximum value at pH 8.2. Any further increase in the pH above that value has affected a graduate decrease in the absorbance at 420 nm , meanwhile a graduate increase in the absorbance at 520 nm has been observed. This increase was due to the dissociation of HA^{2-} to

A^{3-} which was found to absorb at 520 nm. The absorbance at 520 nm reached its maximum value at pH 12. Any further increase in the pH had no influence on the absorbance.

Stability of the color :

The stability of the color of emodin has been studied spectrophotometrically at 520 nm and at pH 12. It was found that the color is reached its maximum value within one minute after the addition of the buffer solution and it remains constant for more than 24 hours.

Beer's law and sensitivity :

Following the general procedure , a linear relationship was obtained between the absorbance and emodin concentration within the range of 2-30 ppm. From the calibration curve the molar absorbtivity was calculated to be 8.86×10^3 $\ell \cdot mol^{-1} \cdot cm^{-1}$. and the relative standard deviation for 16.2 ppm. was 1.4 % for 5 measurements.

Applications :

The method was applied for the determination of emodin in roots , stems, and leaves of Rumex cyprius. The samples were prepared as described in the recommended procedure. The results obtained showed that the concentration of emodin in roots , stems and leaves were 108 ppm , 13.5 ppm and 297 ppm in dried plant samples respectively.

Acknowledgment

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