SPECTROSCOPY LETTERS, 27(4), 409-416 (1994)

PLUMBAGIN , A NATURALLY OCCURRING NAPHTHOQUINONE: ITS ISOLATION , SPECTROPHOTOMETRIC DETERMINATION IN ROOTS , STEMS , AND LEAVES IN PLUMBAGO EUROPAEA L.

KEY WORDS : Plumbagin, Isolation, Spectrophotometric Determination, Roots, Stems, Leaves, Plumbago Europaea L. , pK_a , Acid-Base Indicator.

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ABSTRACT

A new method for isolation and spectrophotometric determination of plumbagin is presented. Plumbagin was isolated by thin layer chromatography (TLC) and column chromatography (CC) techniques , as an orange tinged yellow long crystalline substances. Plumbagin exhibits two absroption maxima at 410 and 510 nm. Stability of the color , pK_a value , and the effect of pH were studied. Beer's law is obeyed over the range 0.9 - 45 ppm. The method is applied to the determination of plumbagin in roots , stems , and leaves of Plumbago europaea L. plant.

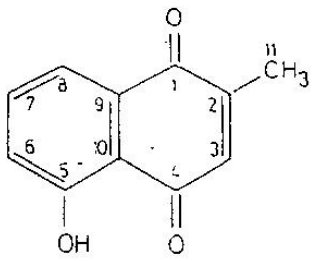
INTRODUCTION

Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) I , a naturally occuring naphthoquinone found in Droseraceae , Ebenaceae , and Plumbaginaceae , has a multivarious effects in pharmacology and pest control (1).

Plumbago europaea L. (Plubaginaceae) is considered as a traditional age-old medicinal plant used in folk medicine for treatment of skin diseases in the West Bank of Jordan.

Many methods had been used for determination of plumbagin in different parts of Plumbago europaea L. (2).

In the present work , a new method has been described for isolation and spectrophotometric determination of plumbagin in roots , stems , and leaves of Plumbago europaea L.



Plumbagin I

EXPERIMENTAL

Chemicals and Reagents :

Plumbagin 0.01 M. The proper weight of plumbagin was dissolved in 25 ml of ethanol.

Buffer solutions. The buffer solutions in the pH range 2.0 - 13.0 were prepared from acetic acid - boric acid - phosphoric acid and sodium hydroxide mixtures.

Apparatus. A Pye-Unicam SP 8-100 spectrophotometer was used with a quartz cell (1X1 cm). All measurements were performed at room temperature (22 °C).

General Procedures :

Separation of plumbagin by column chromatography :

The plant material was collected in Spring , dried , and divided into three parts : roots , stems , and leaves. 10 g of each part was extracted in 95% ethanol for 24 hours in the soxhlet extractor. Ethanol was evaporated in vacuo. The residue was subjected to column chromatography using silica gel and diethyl ether - petroleum ether (60 - 80°) [1 : 1] as eluent. Plumbagin was obtained in a pure form as an orange tinged yellow long crystalline substance , with an R_f = 0.86 and m.p. 78-79 °C (3,4,5).

Thin layer chromatographic separation of plumbagin :

The thin layer chromatography method has been used for separation of Plumbago Europaea L. components. Three spots were detected under UV light (362 nm) having yellow , green , and blue colors on the chromatogram

with R_f values of 0.86 , 0.65 , and 0.5 respectively. Plumbagin was separated and identified using spectroscopic analysis (5).

Upon spraying the dry chromatogram with buffer solution pH 12 , the plumbagin spot was the only spot which gave a violet color , while the other components did not show any change in colors.

Spectrophotometric determination of plumbagin :

A portion of solution containing an amount of plumbagin within the range 4.5 - 224 µg was transferred into a 5-ml volumetric flask. The volume was completed with the buffer solution pH 12 and the absorbance was measured after 2 minutes at 510 nm against buffer solution as a reference.

RESULTS AND DISCUSSION

Absorption spectra :

The absorption spectra of plumbagin were studied in the wavelength range 300 - 700 nm and in the pH range 2.00 - 12.00 . The results obtained showed that plumbagin exhibits two absorption maxima at 410 nm and at 510 nm as shown in Figure 1. The absorption peak at 410 nm was found to decrease gradually by increasing the pH , while the absorption peak at 510 nm was found to increase gradually by increasing the pH. These observations confirmed that the absorption peak at 410 nm was due to the acidic form of plumbagin while the absorption peak at 510 nm was due to the basic form of plumbagin.

Effect of pH :

The effect of pH on the absorbance of plumbagin was studied in the pH range 2.00 - 13.00 . The results obtained (Fig. 2) showed that

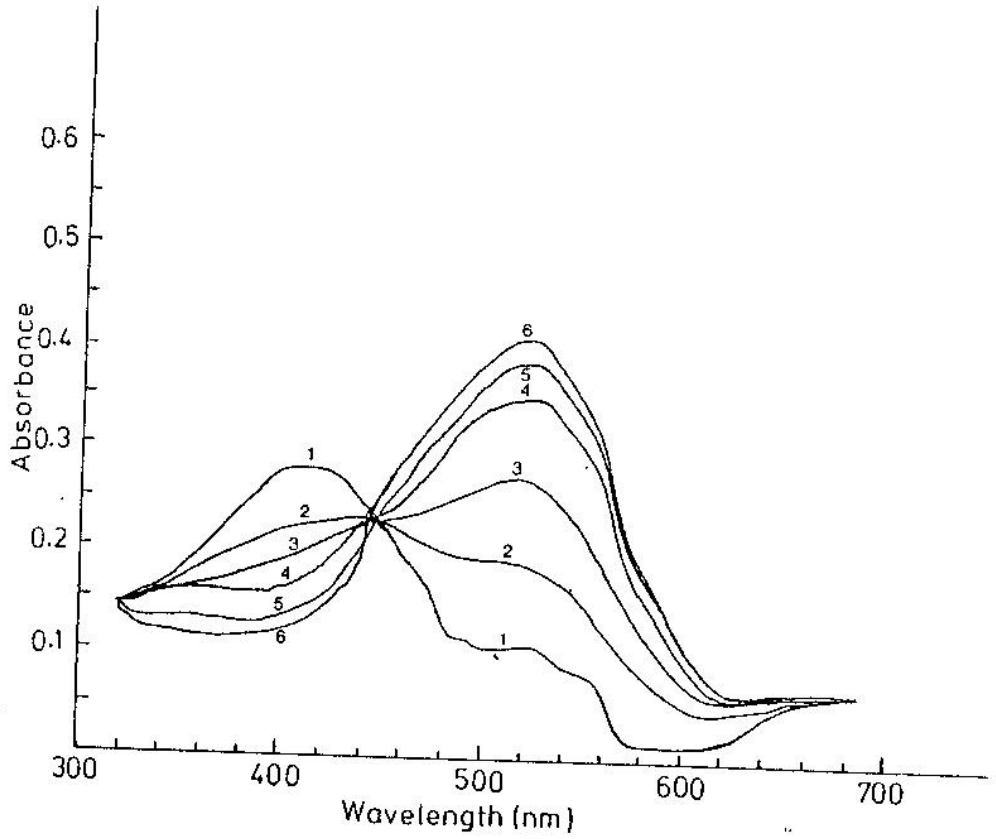


Fig. 1 . Apsorption spectra of 10^{-4} M plumbagin at various pH values. 1- 7.95 ; 2- 8.85 ; 3- 9.55 ; 4- 10.00 ; 5- 11.00 ; 6- 12.00 .

increasing the pH in the range (2.00 - 8.00) did not affect the absorbance. Any further increase in the pH affects a gradual increase in the absorbance up to pH 10.00 beyond which any further increase in the pH did not affect the absorbance as shown in Figure 1.

Stability of the color :

The color of plumbagin is attained within 2 minutes after the addition of the buffer solution and it remains constant for at least 24 hours.

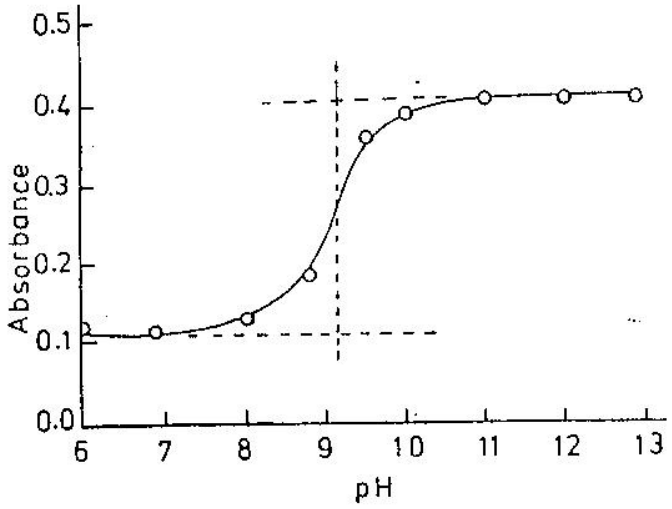


Fig. 2. Absorbance of 10^{-4} M plumbagin at λ = 510 nm as a function of pH.

The pK_a of plumbagin :

Refering to Figure 1 , the absorbance of 10^{-4} M plumbagin at 510 nm was measured at different pH values and the results obtained were presented in Figure 2. It can be seen from Figure 2 that plumbagin has a pK_a value of 9.10 which indicates that plumbagin can act as an acid-base indicator with a pH transition range 8.00 to 10.00 .

Beer's law and sensitivity :

The factors that affect the color development were studied and the conditions for absorbance measurements were selected. Following the general procedure , a rectilinear relationship was obtained between the absorbance and plumbagin concentration within the range 0.9 - 45 ppm. From the calibration curve the molar absorptivity was calculated to be 4.0×10^3 l. mol^{-1}. cm^{-1} and the relative standard deviation for 18.8 ppm was 3.8% for 10 measurements.

Applications :

The method was applied for the determination of plumbagin in roots , stems , and leaves of Plumbago europaea L.

The samples were prepared as described in the general procedure. The results obtained showed that the concentration of plumbagin in roots , stems , and leaves were 0.100% , and 0.560% , and 1.500% in dried plant sample respectively.

ACKNOWLEDGMENT

The authors would like to thank AMIDEAST agency for financial support.

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Date Received: January 5, 1994
Date Accepted: February 11, 1994