TLC-Colourimetric Estimation of Free and Combined forms of Chrysophanol, Emodin and Phycione in Indian Rhubarb.

Khan Mohib and M. S. Shingare*
Department of Chemistry, Dr. B. A. M. University, Aurangabad-431 004.

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TLC-colourimetric method was employed for the estimation of free and combined forms of chrysophanol, emodin and phycione in Indian rhubarb. It was found that Indian rhubarb contains free chrysophanol (0.15%), O-glycosidic chrysophanol (0.06%), C-glycosidic chrysophanol (0.21%), free emodin (0.07%), O-glycosidic emodin (0.30%), C-glycosidic emodin (0.08%), free phycione (0.40%), O-glycosidic phycione (0.18%) and C-glycosidic phycione (0.17%).

Indian rhubarb consists of dried rhizomes of Rheum emodi, Rheum webbianum and other species of Rheum family Polygonaceae. It is collected usually from 6-7 years old plant before the flowering season and marketed with cortex intact or partially decorticated. It is purgative, diuretic and is used in a number of diseases and disorders.

Chrysophanol is 1, 8-dihydroxy-3-methyl anthraquinone. It occurs in free and combined states in cascara, senna and rhubarb. Emodin is 1, 3, 8-trihydroxy-6-methyl anthraquinone. It occurs in free and combined states in rhubarb, cascara and rumex. Phycione is 1, 8-dihydroxy-3-methoxy-6-methyl anthraquinone. The free anthraquinone derivatives produce pink, red or violet colour with aqueous ammonia or caustic soda solution. As these anthraquinones, chrysophanol, emodin and phycione are quite interesting it was considered worthwhile to estimate them in their free and combined form in Indian rhubarb by TLC-colourimetric method and hence the present studies were taken up. There are also other methods for the estimation of anthraquinones like fluorimetry and high performance liquid chromatography.

The research material was collected from Shabbar Dawasaaz Pan Dariba Aurangabad and authenticated as Indian rhubarb. It was and used for the estimation. The following samples were prepared for the estimation of anthraquinones. Free form was prepared from accurately weighed and powdered 1 g quantity of the drug. It was extracted by refluxing with 50 ml of chloroform for 4 h. It was then filtered. The filtrate was then concentrated to about 1 ml and used for the determination of free chrysophanol, emodin and phycione. (E-1). The marc left after filtration was used for the next step. The combined-O-form was prepared from the marc left after exhaustion with chloroform, which was heated with 50 ml of dilute sulphuric acid for 4 h. It was then filtered. The filtrate was extracted two times with 50 ml of chloroform and the combined chloroform extracts concentrated to about 1 ml. The concentrate was used for the determination of free chrysophanol, emodin and phycione, which were present in combined-O-form (E-2). The marc left after filtration was used for the next step.

The combined-C-form was prepared from the marc left after exhaustion with dilute sulphuric acid. This marc was heated with 50 ml of dilute sulphuric acid and 5 g of ferric chloride for 4 h. It was then filtered. The filtrate was extracted two times with 50 ml of chloroform and the combined chloroform extracts concentrated to about 1 ml. The concentrate was used for the determination of free chrysophanol, emodin and phycione the constituents of the combined-C-form (E-3).

The 1 ml chloroform concentrates (E-1, E-2 and E-3) of Indian rhubarb were subjected to thin layer chromatography. Along with the row of spots, one spot of each authentic compound (chrysophanol, emodin and phycione) was also spotted with the help of capillary. The solvent system used for the development of the plates was Petroleum ether: Ethyl acetate; Formic acid (75:25:1). The solvent system was allowed to run up to 15 cm from the point of application of the

*For correspondence
spots. The plates were removed from the chamber and air-dried. The all three compounds, chrysophanol, emodin and physcion were coloured and had shown Rf values as chrysophanol (0.6), emodin (0.4) and physcion (0.7). All corresponding bands were scrapped out with the help of sharp blades and collected individually in separate centrifuge tube.

The stock solutions of chrysophanol, emodin and physcion separately were prepared by dissolving 10 mg each of the anthraquinone in aqueous potassium hydroxide solution in three separate volumetric flasks. From the stock solutions, 0.2, 0.4, 0.6, 0.8 and 1.0 ml were transferred to clean dry tubes. Then 9.8, 9.6, 9.4, 9.2 and 9.0 ml of distilled water were added to give working dilutions of 20, 40, 60, 80 and 100 μg per 10 ml. The working dilutions were set aside for 15 min and their optical density were measured on colourimeter at 480 nm against 1N aqueous potassium hydroxide solution as a blank. The standard curves were prepared by plotting optical density against concentration in μg per 10 ml.

The compounds, chrysophanol, emodin and physcion, embedded in silica gel G, were extracted with chloroform. The extracts were collected in different petri dishes and evaporated to dryness. Then 10 ml of 1 N potassium hydroxide solution and thoroughly stirred for 10 min. The optical densities of all the compounds were measured on colourimeter at 480 nm against IN aqueous potassium hydroxide solution as a blank. The calibration curves for chrysophanol, emodin and physcion were found to obey Beer's law.

It was found that Indian rhubarb contains free chrysophanol (0.15 %), O-glycosidic chrysophanol (0.06 %), C-glycosidic chrysophanol (0.21%), free emodin (0.07 %), O-glycosidic emodin (0.30 %), C-glycosidic emodin (0.08 %), free physcion (0.40 %), O-glycosidic physcion (0.18 %) and C-glycosidic physcion (0.17 %).

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