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Qualitative and Quantitative Study of Anthraquinone Derivatives in the Root Extract of *Rheum australe* of Nepal (syn. *Rheum emodi*) by HPLC

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ABSTRACT

High performance liquid chromatography was applied for the separation and identification of four anthraquinone derivatives, aloe emodin, emodin, chrysophanol and physcion from the root extracts of *Rheum australe* (syn. *Rheum emodi* WALL, Polygonaceae) collected from Gyan herbal products Nepal. The separation was carried out using, C18 column (4.6 mm i.d. x 250 mm, 5 µm) under the following conditions: Acetonitrile and water: acetic acid (75:20:5, pH 3.5) as a mobile phase with isocratic elution at flow rate 1 ml/min. The detection wavelength was set at 254 nm. *Rheum australe* root extract of Nepal has shown higher percentage concentrations of emodin and physcion than aloe emodin, chrysophanol and rutins as 15 %, 4.2 %, 0.46 %, 1.6 % and 0. 9% > 0.05 S.D. This method can be used for both qualitative and quantitative determinations of total anthraquinone contents in the standardization of Rhubarb extracts.

Keywords: *Rheum australe*, Anthraquinone Derivatives, Root Extract.

1. Introduction

The roots of *Rheum australe* (syn. *Rheum emodi* WALL, Polygonaceae) are widely used in Ayurvedic and Asian folk medicine. Rhubarb is the English name of the genus Rheum Linn (family polygonaceae). There are about 60 species of the genus Rheum recorded in the world [1]. Studies have reported that extracts from rhubarb have different medicinal activities such as purgative [2], antimicrobial [3], antispasmodic [4], antidiabetic [5], antiviral [6], and antioxidant [7]. Rheum australe a variety of Rheum family found at various methods for anthraquinone derivatives identification and quantification in plant extracts have been reported such as high-speed counter-current chromatography high performance thin layer chromatography and most commonly used high performance liquid chromatography HPLC [8, 9]. Hence, in the present study, we report, a simple analytical method using HPLC for qualitative and quantitative determination of four anthraquinone derivatives namely aloe-emodin, emodin, chrysophanol and physcion in the roots of *Rheum australe*. Pharmacologically, besides the purgative effect which is widely known [10], this genus has antimicrobial, anti-tumor and anti-inflammatory activities.

In an effort to develop an improved method for the determination of anthraquinone content in *Rheum australe* root extract and its quality control, an extraction method with simultaneous quantification of the anthraquinone in *Rheum australe* root through HPLC was developed. The HPLC method was also validated.

2. Materials and Methods

2.1 Sample preparation:

Methanol and water (1:2) extract (0.01 gm) was dissolved in 10 ml of Acetonitrile: water: acetic acid in the ratio of 75: 20: 5. As 1 mg/ml of sample in solvent was prepared and degassed using ultrasonicator.

2.2 HPLC analysis method

The separation was carried out using, C18 column (4.6 mm i.d. x 250 mm, 5 µm) under the following conditions: Acetonitrile and water: acetic acid (75:20:5, pH 3.5) as a mobile phase with isocratic elution at flow rate 1 ml/min. The detection wavelength was set at 254 nm. Sample solution of 20 µl was injected and components were detected at l max = 254 nm using variable wavelength UV detector. Components were identified by comparing standard peaks published as shown in figure 1 and 2 with their retention time and quantified by standard peak area and percentage of concentration method.

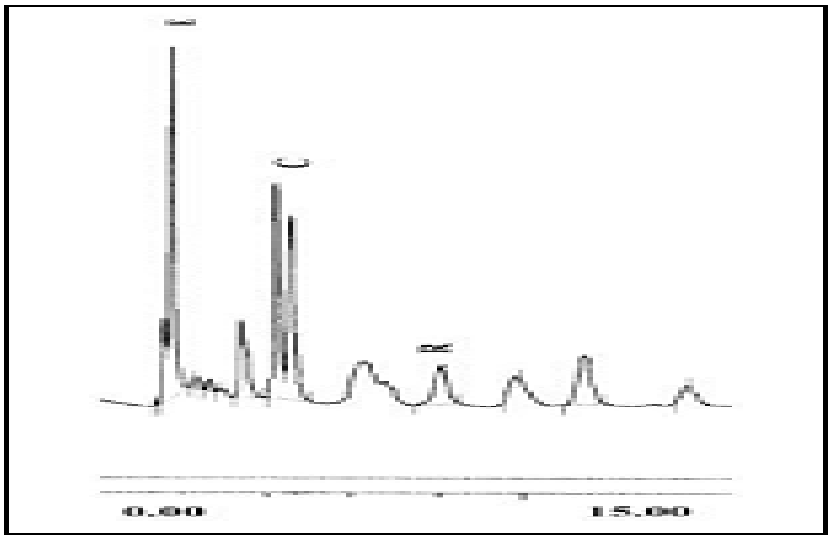

Fig 1: chromatogram of emodin, chrysophanol and rutin (RT 6.1, 7.8, 9.3) obtained from Pankaj Prasad *et al* .current science

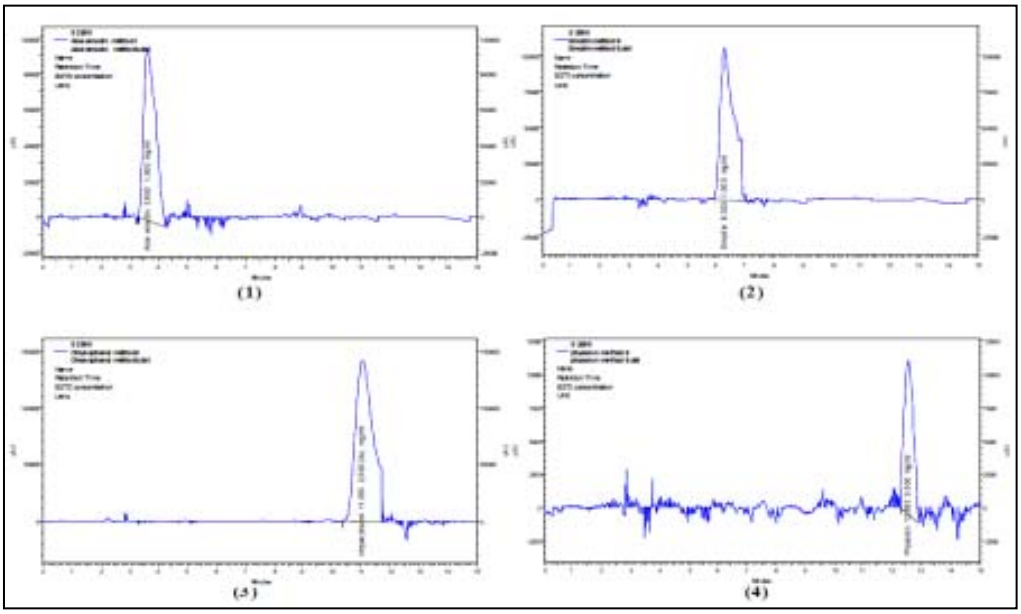

Fig 2: Hplc chromatograms of standards 1.aloe emodin 2.emodin 3.chrysophanol 4.physcion

3. Result and discussion:

We examined the optimal conditions for the simultaneous quantitative determination of Anthraquinones in *Rhubarb* root extract obtained from Gyan herbal products Nepal using an isocratic reversed-phase HPLC system. As all four compounds have good absorption at 254 nm, this wavelength was used for quantization. Mixtures of Acetonitrile, water, acetic acid were examined as the mobile phase and its composition was optimized. The ratio of Acetonitrile, water and acetic acid is 75:20:5. This ratio obtained a good resolution of anthraquinone. All anthraquinone compounds were eluted within 30 min with satisfactory resolution at different runs (Figure 3). *Rheum australe* root extract of Nepal has shown higher percentage concentrations of emodin and physcion than aloe emodin, chrysophanol and rutins as 15 %, 4.2 %, 0.46 %, 1.6 % and 0.9% > 0.05 S.D as in Table 1.

Table 1 Quantitative analysis *Rheum australe* (syn. *Rheum emodi*) extracts by HPLC

Compound name	RT	% Concentration
Aloe emodin	3.58+/-0.05	0.46+/-0.05
emodin	6.4+/-0.05	15+/-0.05
chrysophanol	10.5+/0.05	1.6+/-0.05
physcion	11.2+/-0.05	4.2+/-0.05
rutin	9.6+/-0.05	0.9+/-0.05

The proposed isocratic procedure exhibits simultaneous quantification for the anthraquinone analytes (aloe-emodin, emodin, chrysophanol, physcion and rutins) and is simple, rapid, and selective. The previously reported HPLC method required the use of a gradient elution system and required a longer time; a total run time of approximately 60 min is required, and validation of the analytical procedure is not yet established. In addition, the spectrophotometric method requires several time-consuming sample preparation steps with anthraquinone content determined as total
hydroxyanthracene derivative [11].

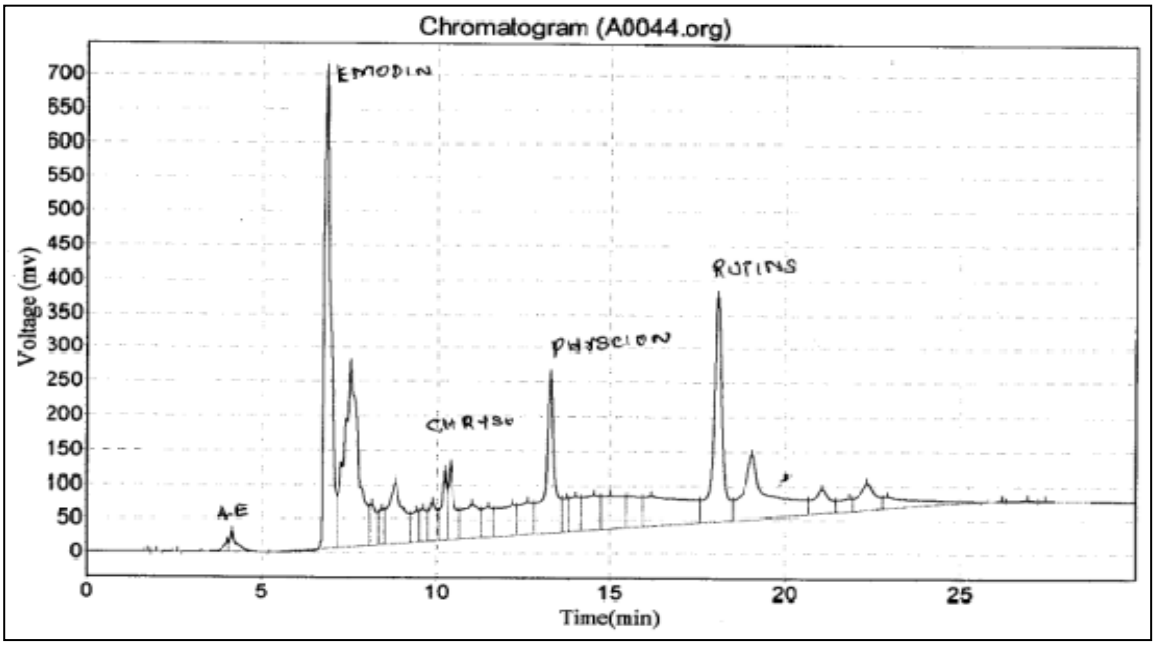

Fig 3: HPLC chromatogram of alcoholic extract with aloe emodin, emodin, chrysophanol, physcion, rutin and other glycosides of *Rheum australe* root. (Can be comparable with standard graphs)

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