

Isolation of Eriocitrin (eriodictyol 7-O-rutinoside) from Orange (*citrus sinuses*) Peel by Using Reverse Phase HPLC

Syeda Mahejabeen¹, P. Nirmala²

¹Research Scholar, Department of Biotechnology, Nehru Arts and Science College, Coimbatore, Tamilnadu, India

²Associate Professor, Department of Biotechnology, Nehru Arts and Science College, Coimbatore, Tamilnadu, India

Abstract: *Orange (Citrus sinuses) is well known for its nutritional and medicinal properties through the world. Citrus belongs to family Rutaceae. In search for anticancer drugs compelling data from laboratories, epidemiologic investigations, and human clinical trials showed that flavonoids have important effects on cancer chemoprevention and chemotherapy. An objective of the present study is to isolate Eriocitrin a flavanone from Orange peel by using Column Chromatography Technique. Orange peel was shadow dried for 7-10 days and defatted using nHexane, then the residue was extracted using Ethyl Acetate at RT overnight. The extract was air dried and suspended in 1ml of ethyl acetate. A silica gel column of size 300mm X10mm was packed and the 1ml of ethyl acetate extract was loaded onto the column and eluted by using 25 ml ethyl acetate, each 5ml fractions. All these fractions were pooled and checked for Eriocitrin content by HPLC analysis. Results showed that Concentration of Eriocitrin in orange peel powder was found to be 31.57µg/ml. Purity of Eriocitrin by HPLC in Ethyl acetate fraction was found 52 %.*

Keywords: Eriocitrin, Column Chromatography, RP-HPLC Analysis, UV absorption at 280nm

1. Introduction

Citrus sinuses peels has been known to be a potential natural antioxidants because of their phenolic and flavonoid compounds. Flavonoids are water soluble polyphenolic molecules containing 15 carbon atoms[1,2]. The biological properties of flavonoids include antioxidant, anti-inflammatory, antitumoral, antiviral and antibacterial, as well as a direct cytoprotective effect on coronary and vascular systems, the pancreas, Breast and the liver [3,4]. These characteristics place them among the most attractive natural substances available to enrich the current therapy options because they also help support detoxification of potentially tissue-damaging molecules, their intake has often, although not always, been associated with decreased risk of certain types of cancers, including lung and

Breast cancer [5]. Flavonoids have been shown to reveal cytotoxic activity toward various human cancer cells with little or no effect on normal cells, and this fact has stimulated large interest in developing of potential flavonoid-based therapeutics for anticancer treatment. Due to the polyphenolic structure, flavonoids have been found to possess both anti- and prooxidant action. While antioxidant effect and ability to scavenge reactive oxygen species (ROS) have been shown to account for most of the reported biological effects of phenolic compounds. In many molecular mechanisms of action for prevention against cancer, flavonoids play a major role by interacting between different types of genes and enzymes [6].

2. Materials and Method

Citrus sinuses were purchased from super market, Silica gel column (300mm x 10mm), Standards: Eriocitrin Standard (Sigma) stock solution (1mg/mL) was prepared in HPLC grade Methanol. Working standard solution: Test

concentrations 25, 50 and 100 µg/mL were prepared from stock solution. Samples: Ethyl acetate fraction (10mg/mL) was prepared from stock and Used for HPLC analysis. Instrument was from Shimadzhu LC- Prominence 20AT, Column: C18 column 250 mm x 4.6 mm, 5µM particle size. GC-MS (Thermo scientific GC Trace 1310 Equipped with Thermo Scientific MS TSQ 8000)

Sample Preparation

The orange peel was shadow dried for 7-10 days. 100g of powdered orange peel was weighed and extracted with 500ml of nhexane for 4 hours at RT in a magnetic stirrer maintained at 2000rpm. The extract was filtered using 0.4µM filter and the residue was air dried at RT. The residue was extracted by using 500ml of Ethyl acetate overnight (16 hours) at RT in a magnetic stirrer at 2000 RPM. The extract was evaporated to dryness and reconstituted in 1ml of Ethyl Acetate [7, 8]

Column Purification

1ml of the concentrated Ethyl Acetate extract was loaded onto the silica gel column (300mm x 10mm). The column was washed with 25 ml of hexane and 5ml fractions were collected [9]. Elution was done using 25ml of ethyl acetate and 5ml fractions were collected. Then the 5ml fractions were pooled and checked for Eriocitrin content by using reverse phase HPLC [10].

HPLC Analysis

Ethyl acetate fraction (10mg/mL) was prepared from stock and Used for HPLC analysis by using a C18 reverse phase column (RP-18, 250mmx 4.6mm, 5µM) with a linear gradient. The solvent system contained mobile phase A-0.1% formic acid in acetonitrile (20%) and mobile phase B-0.1% formic acid in HPLC water (80%). The injection volume was 10 µL and the flow rate was 1mL per min and absorbance was monitored at 280 nm.

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3. Results and Discussion

HPLC conditions were carried out for separation of Eriocitrin. HPLC chromatograms of standards and ethylacetate fraction are shown in Figure 2-6. Eriocitrin content in the unknown fractions of orange peel were determined using the regression equation $Y = 5.201 x + 12.73$, derived from standard Eriocitrin response (Table 1), Where Y = Area of peak (mV.S), X = Concentration of eriocitrin in $\mu\text{g/mL}$.

$$X = 12.73 - 176.996 / 5.201 = 31.57$$

$$\mathbf{X} = 31.57 \text{ } \mu\text{g/mL}$$

Quantification

The retention time of samples were matched with that of each standard and concentration of respective standard were calculated using the formula.

$$\text{Eriocitrin (Microgram/mL)} = \frac{\text{Sample area}}{\text{Standard area}} \times \text{Standard concentration injected} \times \text{Dilution factor}$$

4. Conclusion

From the above procedural workout, it can be clearly concluded that the *Citrus sinensis* does contain the flavonoid Eriocitrin, HPLC analysis further confirms the presence of Eriocitrin. Results showed that Concentration of Eriocitrin in orange peel powder was found to be 31.57 μ g/ml. Purity of Eriocitrin by HPLC in Ethyl acetate fraction was found 52 %. This procedure, hence therefore though being a very simple process and efficient for the purification of compounds from crude extracts of the plants. The presence of Eriocitrin in peel of citrus sinuses should facilitate the effective utilization of this compound.

Tables and Chromatograms

Table 1: HPLC summary report of Standard

S No	Standard concentration ($\mu\text{g/mL}$)	Retention time (min)	Area of Eriocitrin (mV.S.)	Chromatogram references
1	Solvent Blank	-	0	Fig.2
2	25	10.580	167.210	Fig.3
3	50	10.590	261.636	Fig.4
4	100	10.650	532.406	Fig.5

Table 2: HPLC summary Report of Orange peel

Sl. No	Sample (concentration 10 mg/mL)	R.T (min)	Area of (mV.s)	concentration ($\mu\text{g/mL}$)	Chromatogram references
1	Ethyl acetate fractions	10.080	176.996	31.57	Fig.6

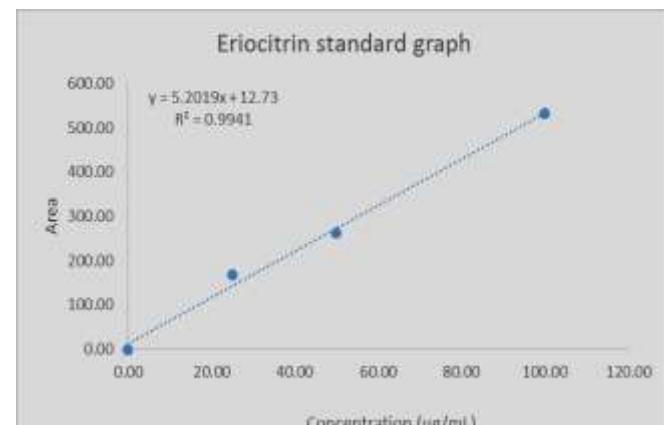


Figure 1: Standard curve of Eriocitrin

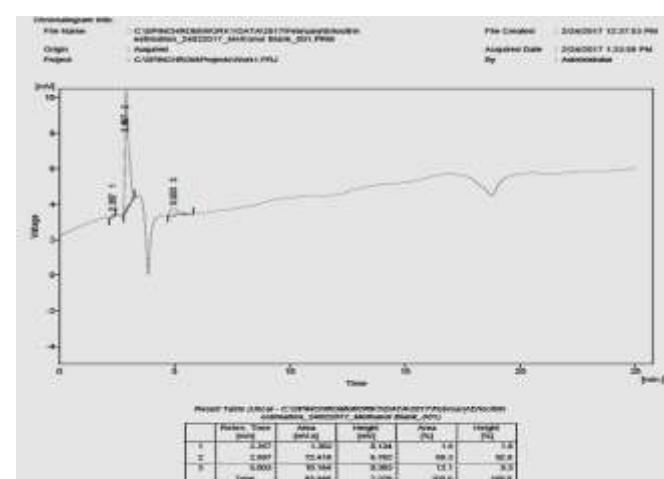


Figure 2: HPLC chromatogram of methanol (blank)

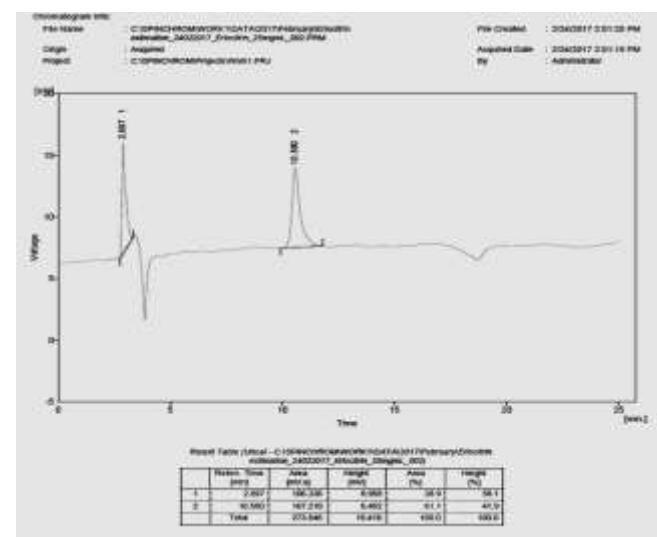


Figure 3: HPLC chromatogram of standard concentration
25 µg/mL

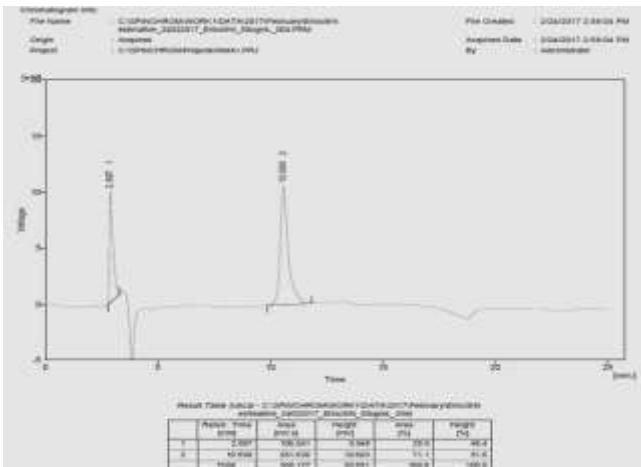


Figure 4: HPLC chromatogram of standard concentration 50 µg/mL

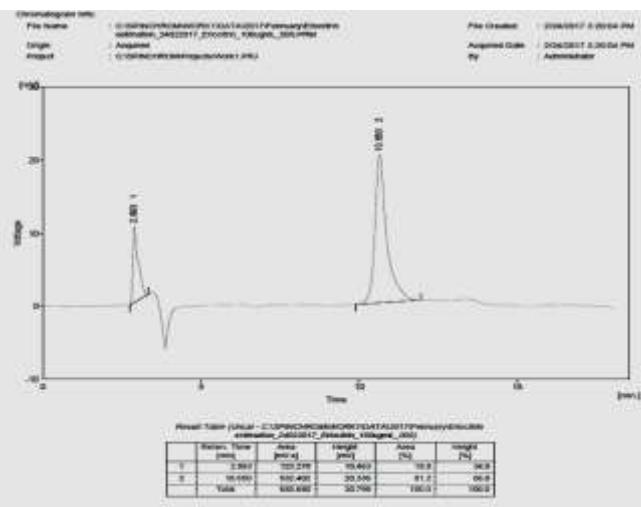


Figure 5: HPLC chromatogram of standard concentration 100 µg/mL

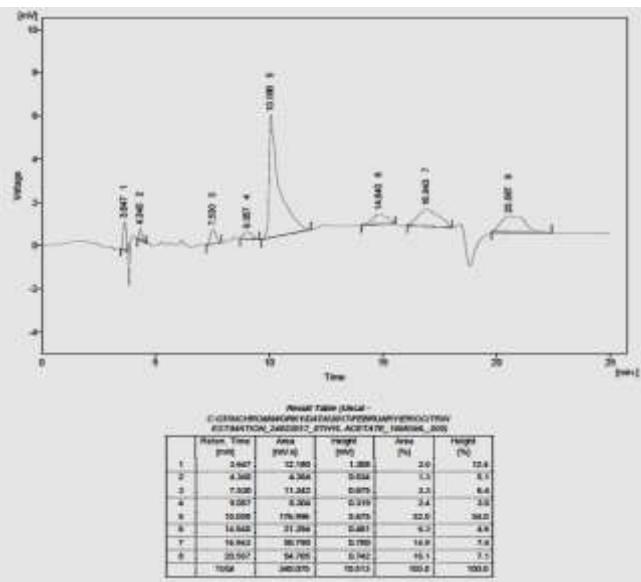


Figure 6: HPLC chromatogram of ethyl acetate fraction (10 mg/mL)

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