

## Isolation and Identification of Flavonoids in Iraqi *Rheum ribes* Root

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### ABSTRACT

The aim of the study was isolate and identify the flavonoids in Iraqi *Rheum ribes* root, the results obtained from the HPLC analysis indicate that the Iraqi *Rheum ribes* root contain different types and concentration of flavonoids which include 29.9056 µg/g aloeo-emodin, 252.880 µg/g emodin, 76.8493 µg/g chrysophanol, 56.1317 µg/g physcion and 49.2098 µg/g rutin, while the types of flavonoids in the isolated extract of flavonoids from the Iraqi *Rheum ribes* root were 146.096 µg/g aloeo-emodin, 37.174 µg/g emodin, 11.525 µg/g chrysophanol, 20.010 µg/g physcion, 30.271 µg/g rutin. The identification the all types of flavonoids in isolated flavonoids from the Iraqi *Rheum ribes* roots as compared with the flavonoids in the crude roots refers to the efficiency of the method for isolation of flavonoids from the roots of plant.

**Keywords:** *Rheum ribes* roots, flavonoids, HPLC analysis, Aleo-emodin, emodin, chrysophanol, physcion, rutin

### INTRODUCTION

Thousands of newer compounds and organic molecules in phytochemistry has been identified every year, different isolation methods, Pharmacological testing, biochemical methods were used to discovering and developing new drugs from secondary metabolite in plants, which used as drugs or to prevent various diseases, this compounds includes flavonoids, alkaloids and terpenoids, etc. [1,2].

Flavonoids are low molecular weight [3], bioactive poly-phenols [4], widely distributed among the plant kingdom, which play a vital role in photosynthesising cells [5]. Flavonoids have been reported to exert a wide range of biological activities. These include: anti-inflammatory, antibacterial, antiviral, anti-allergic and anti-oxidant [5,6]. Many types of flavonoids are used to treat common diseases, such as quercetin which used to treat herpes virus, and also to reduce the plasma bad fat [Low density lipoprotein-cholesterol] [7], while Cyandidanol-3, meciadanol and catechins used to treat Ulcer by its action to Gastric H<sup>+</sup>/K<sup>+</sup> ATPase [8], also Genistein and Quercetin have anticancer effect [9], Fisetin and Quercetin used as anti-diabetes [10].

*Rheum ribes* Linn (Polygonaceae) is widely distributed in the north of Iraq, Turkey, Iran, Afghanistan, Russia and Pakistan. Young shoots of the plants and petals are eaten as a vegetable in Iraq and also used to improve appetite and treat diarrhea [11,12]. Roots of the plants are widely used in traditional medicine to treat ulcers, diarrhea, hemorrhoids and reported to have an antidiabetic effect [13], antibacterial activity [14] and antioxidant properties [15] due to the present of phenolic compounds (good natural antioxidants) [16], and also flavonoids such as Aloe-emodin, Emodin, Rhein, Chrysophanol, Kaempferol, ..., etc. [15].

So that, the present study aims to isolate flavonoids from the roots of the plants, and evaluate the isolation method by identify the types of flavonoids in the isolated part and compare with flavonoids in crude roots.

## MATERIAL AND METHODS

The general laboratory chemicals with highest purity are used in this study, which obtained from Sigma-Aldrich, BDH and GCC.

### Plant Collection:

The *Rheum ribes* Linn roots were obtained from the local market of Kirkuk in north of Iraq. Authentication was done at the herbarium of the Faculty of Science, Tikrit University.

### Extraction of Flavonoids:

The extraction of flavonoids from *Rheum ribes* was done according to the method of (Chen *et al*, method) with some modification [17]. Firstly must remove the fatty contents by using soxhlet apparatus, in which 50g of the dried roots powder were extracted with 150ml of diethyl ether for 3 hours. The defatted powder was dried at 40°C in an air oven, and then extracted twice with condenser by using an ethanol solution as solvent for extraction [200ml (70%)] at 90°C for 2h, filtration and then centrifugation at 3000 rpm for 15 min, evaporated the solvent and the aqueous extract was condensed under reduced pressure and then the extract was stored at 4°C until used.

### Quantitative determination of total flavonoid:

The quantitative determination of flavonoids in dried roots of *Rheum ribes* was done according to the aluminum chloride method (colorimetric method) [18].

### Analysis of Flavonoids by HPLC:

## Sample Preparation

One gram of *Rheum ribes* Linn roots powder was dissolved in 5ml of 80% ethanol in a test tube, mixed and used ultrasonic for 25min at 25°C, and then the supernatant was obtained after centrifugation at 7500rpm for 15min, after that the charcoal was used to removed dye from the extract, The solvent was evaporated to dryness by rotary evaporator at 35°C, and then dissolved with 1ml of ethanol and mixed well by vortex, 20µl was subjected to analysis by HPLC.

## Method of Analysis

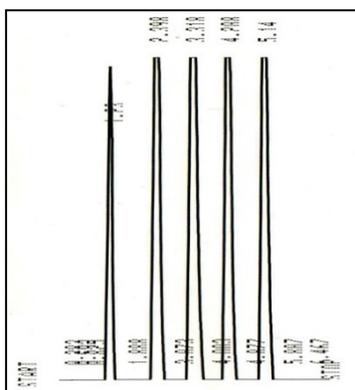
The sample flavonoids for the roots and for the isolated flavonoids were done according to the method of [19,20] with some modification, by using C-18 column (250 × 4.6 mm). The mobile phase consisted of linear graduated of solvent A(0.1% formic acid), solvent B(1:3:6)v/v acetonitrile, methanol and 0.1%formic acid, with flow rate1.2 ml/min and the column temperature was kept at 40°C. The UV detection wavelength was 264 nm.

Five standard solutions was used (25µg/ml), this include Aloe-emodin, Emodin, Chrysophanol, Physcion and Rutin.

## RESULTS AND DISCUSSION

The results indicate that the total flavonoid content in Iraqi *Rheum ribes* Linn roots was 22.680mg/gm which assessed by aluminum chloride method. This result was more than the results of Ibrahim et al. [21], which found that the ethanolic extract of *Rheum ribes* roots contain 687mg/100g of flavonoids and 149.01mg/100g in water extract.

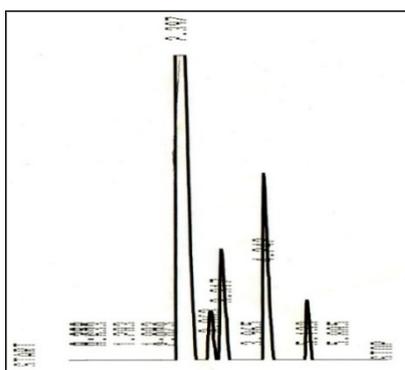
The analysis of flavonoids by HPLC in Iraqi *Rheum ribes* Linn roots and its isolated flavonoids was carried out, **Figure 1** showed the peaks of the standard flavonoids, with different Retention times -Rt, **Table 1**.



**Figure 1.** HPLC Analysis of 5 standard flavonoids

**Table 1.** Retention Times and Area Under Curves for Standard Flavonoids

Standard	Rt (min)	Area
Aloe-emodin	1.23	62348
Emodin	2.39	117329
Chrysophanol	3.31	138104
Physcion	4.20	110687
Rutin	5.14	105869



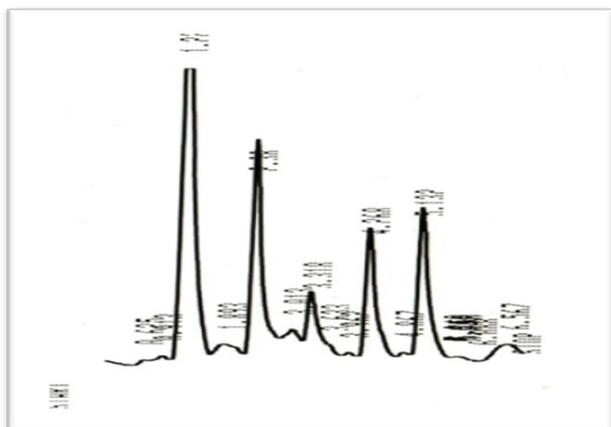
**Figure 2.** HPLC Analysis of flavonoids in crude Iraqi *Rheum ribes* roots

**Table 2.** Retention Times and Area Under Curves for Iraqi *Rheum ribes* roots flavonoids

Rt.(min)	Area	Identified compounds	Conc. (µg/g)
1.203	15538	Aloe-emodin	29.9056
2.397	247252	Emodin	252.880
3.063	35603	Unknown	-
3.317	53066	Chrysophanol	76.8493
3.965	11595	Unknown	-
4.242	60077	Physcion	56.1317
5.133	43415	Rutin	49.2098
5.805	10488	Unknown	-

The chromatogram of the flavonoids in crude roots was showed eight peaks **Figure 2**, with different  $R_t$  (1.203, 2.397, 3.063, 3.317, 3.965, 4.242, 5.133 and 5.805) min and the area for each peak were (15538, 247252, 35603, 53066, 11595, 60077, 43415 and 10488) which summarized in **Table 2**.

While the chromatograms of isolated flavonoids from the roots of *Rheum ribes* was shown in **Figure 3**, in which ten peaks of flavonoids were obtained with different  $R_t$  (1.23, 1.888, 2.398, 3.078, 3.318, 4.003, 4.208, 4.877, 5.14 and 5.807) min and the area under curves were (62348, 15119, 117329, 14506, 138104, 17186, 110687, 15595, 105869 and 22566).



**Figure 3.** HPLC Analysis of Isolated Flavonoids from *Rheum ribes* roots

**Table 3.** Retention Times and Area Under Curves for isolated flavonoids from Iraqi *Rheum ribes* Linn roots

Rt. (min)	Area	Identified compounds	Conc. ( $\mu\text{g/g}$ )
1.23	62348	Aleo-emodin	146.096
1.888	15119	Unknown	-
2.398	117329	Emodin	37.174
3.078	14506	Unknown	-
3.318	138104	Chrysophanol	11.525
4.003	17186	Unknown	-
4.208	110687	Physcion	20.010
4.877	15595	Unknown	-
5.14	105869	Rutin	30.271
5.807	22566	unknown	-

The results obtained from chromatograms of the crude roots and for the isolated flavonoids as compared with chromatogram of five standard flavonoids, as shown in **Figure 1** and its  $R_t$  value in **Table 1**, indicate that crude roots may contain 29.9056  $\mu\text{g/g}$  aloe-emodin, 252.880  $\mu\text{g/g}$  emodin, 76.8493  $\mu\text{g/g}$  chrysophanol, 56.1317  $\mu\text{g/g}$  physcion and 49.2098  $\mu\text{g/g}$  rutin. The other peaks were unknown, which may indicate to other types of flavonoids, so that we need to use more modern techniques such as HPLC-MS, GC-MS, or NMR or another type of flavonoids for HPLC analysis to identify all types of flavonoids in the roots.

Abdulla et al. [15], identified a mixture of flavonoids in the roots of Iraqi *Rheum ribes* Linn including emodin, aloe-emodin, physcion, chrysophanol and rutin, while Naqishbandi et al. [22] identified only four anthraquinone derivatives which include aloe emodin, emodin, chrysophanol and physcion, but in Turkish roots [23], aloe-emodin, physcion-8-O-glucoside, physcion, chrysophanol, aloe-emodin-8-O-glucoside, rhein, rhaponticin and sennoside A have been identified.

The all types of flavonoids in crude roots were identified in isolated flavonoids, which include 146.096  $\mu\text{g/g}$  aloe-emodin, 37.174  $\mu\text{g/g}$  emodin, 11.525  $\mu\text{g/g}$  chrysophanol, 20.010  $\mu\text{g/g}$  physcion, 30.271  $\mu\text{g/g}$  rutin and four unknown peaks. No information were available in the literature about the isolation or extraction of flavonoids from the roots of *Rheum ribes*, but this method of extraction was widely used to isolate of flavonoids with high yield, which may use in experimental study to identify its effect as anticancer, antidiabetic, antioxidant, antihyperlipidemic, ..., etc., Al-Salihi et al. [24], extracted and isolated flavonoids from Iraqi date palm pollen by the same method used in the present study, and then study the hypolipidemic effect of the isolated flavonoids.

The identification the all types of flavonoids in isolated flavonoids from the Iraqi *Rheum ribes* roots as compared with the flavonoids in the crude roots refers to the efficiency of the method for isolation of flavonoids from the roots of plant.

## CONCLUSION

The anti-oxidant properties of *Rheum ribes* roots, may be due to the high contain of flavonoids, which identified by HPLC. These types of flavonoids include aloe-emodin, emodin, chrysophanol, physcion and rutin which identified in crude roots or in isolated flavonoids from the roots. So that the isolation method, consider an appropriateness method for extraction of flavonoids from *Rheum ribes* roots.

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