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Identification and quantitative analysis of quercetin and luteolin polyphenol in methanolic extracts of *Cirsium arvence* with HPLC

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Polyphenols are important compounds playing an important role in plants and human body. These compounds have an antioxidant role in photosynthesis and they prevent from cancer, cardiovascular disease and diabetes. Luteolin and quercetin are two flavonoid compounds. Flavonoids are found in most plants and used in the pharmaceutical industry. In this study, it was tried to measure the amounts of these two compounds in the aerial organs of C.arvence. HPLC method was used as the analyzing tool of this project. Quercetin and luteolin both are found in two samples of the plant C.arvence collected of two different parts of Iran; A (Neyshabour) and B (Boojnoord). Quercetin were (0.038, 0.113 mg/gr, respectively) and luteolin were (0.233, 0.116 mg/gr, respectively).

Keyword: Cirsium Arvence, HPLC, Quercetin, Luteolin, Flavonoid

INTRODUCTION

Flavonoids are important polyphenolic compounds showing antioxidants role in plants and animals. They prevent form oxidation of macromolecules, lipids and proteins. These compounds are known as antioxidants and can prevent from the onset of various diseases including cancer, heart diseases, diabetes [1,2]. The antioxidant feature of flavonoids is resulted from their chemical structure, which enables them to neutralize free radicals, form metal complex ions. They are deactivated oxygen molecules [3]. Plants are rich sources of antioxidant compounds [4]. Quercetin and luteolin are two important flavonoid compounds. Quercetin can be abundantly found in foods like vegetables, tomatoes and broccoli. As human age increases, quercetin plays more important role in combating free radicals. Today, quercetin is considered as a natural cancer-preventive agent, and as an antioxidant, it can be effective in avoiding cell mutations, growth and proliferation of cancer cells, and also prevent side effects of common cancer treatments such as chemotherapy and radiotherapy [5]. Similar to Quercetin, Luteolin has a high antioxidant, as well as antibacterial and antifungal effects [6,7]. Luteolin is a flavone, a type of flavonoid. Luteolin can be used in health supplements to promote healthy blood glucose levels, as a potent hypoglycemic agent to improve insulin sensitivity and for helping in weight management for Syndrome X. Luteolin can be used as an antioxidant, anti-inflammatory, anti-allergic and anticancer agent, and its immune-modulating properties can be applied in suppressing hyperactive immune system [8,9]. C.arvense is a medicinal plant. Itbelongs to Asteracease family. This family includes approximately 250-300 species, 28 of which can be found in Iran [10]. Canada (or Canadian) thistle is the common name for C. arvense. It is native to Europe, and can be found in areas of Eastern Mediterranean, West Asia and North Africa [11]. C. arvense is known as "kharlateh" or "kangarSahraee" in Iran. This plant is often found along river banks and in grass lands, and is regarded as a “noxious” weed. The presence of this plant is a sign of soil fertility [12]. The stems of C. arvense are branched in its terminal sections. Its leaves are alternate, lacking petioles, egg- or spear-shaped, with spiny margins which are divided into irregular lobes. Its flower is monosexual, with male and female flowers on separate plants. Its flowers are purple or pink. Although the plant may sometimes not produce seeds, e.g., because of mowing, the existence of productive horizontal, roots that may be cut into smaller parts while plowing, can lead to its rapid multiplication [13,14]. The best-known group of secondary metabolites in Cirsium are flavonoids. For exapmel Rutin was found in Cirsium echinus, C. arvense, and C. undulatum [15,16]. Quercetin was found in C. oleraceum, C. vallis-demonii C. arvense [17]. Kaempferol was found in C. arvense, C. syriacum, C. vallis-demonii [18,19] and Apigenin was found in C. arvense, C. carolinianum, C. japonicum, C. magofukui[20].

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MATERIAL AND METHOD

Chemicals

Methanol, Quercetin(QU), Luteolin (LU), were purchased from Merk Schuchardt (Darmstadt,Germany). Water (DM) was demineralized.

Plant material

The C. arvense (A), used in this study was collected from the Binalood hillside in Neyshabur, Khorasan Razavi at an altitude of 1200 meters above sea level (58º , 47' E – 36º , 12' N) and another (B) was collected from Boojnord, North Khorasan at an altitude of 1071 meters above sea level (57º , 18' E – 37º , 28' N) in May 2015 . The plants were identified by Mr. Joharchi from Department of Botany in Ferdowsi University, Mashhad, Iran.

Preparation of crude extract

The collected aerial organs of the plants material was defatted using *n*-hexane by dynamic maceration for 48 days. After drying, 10 gr of the defatted aerial organs were translated into conical flask (100 mL capacity) containing 50 mL of methanol. It was left for 72 h at room temperature and then filtered, dilution 50 mL in a volumetric flask. Methanol is the most efficient solvent for extract [21-24].

Chromatographic analysis (HPLC)

HPLC analyses were performed with Knauer liquid chromatograph (Netherlands) with two solvent delivery system quaternary pump (Wellchrom HPLC pump K-1001 Knauer) including a UV detector (K-2600 Knauer) with 5 cm flow cell. The column was specification (5 µm particle size, i.d. 4.6 x 250 mm).

Chromatographic conditions

Quercetin and luteolin that obtained from methanol extract of C. arvence was determined by RP- HPLC , as described previously. The mobile phase consisted of methanol (solvent A), Water (solvent B).The flow rate was 1 mL/min. The gradient programming was as follows: Methanol: Water 100:0 for 29 min and 20:80 for 5 min. The injection volume was 20 µl. For UV detection; 365 nm and 343 nm were chosen for quercetin and Luteolin, respectively.

Sample preparation

Standard solution of each phenolic compound were prepared in methanol. For this work 5 mg of the analyte into 100 mL volumetric flask. The mixed standard solution was prepared by dilution the mixed stock standard solutions in methanol to give a concentration of 100µg/mL for each polyphenols. All the standard solutions were stored in the refrigerator at 4º C.

Standard preparation

The calibration curves were made with methanol to yield 7.8 – 100 µg/ml for each phenolic compound.

RESULTS AND DISCUSSION

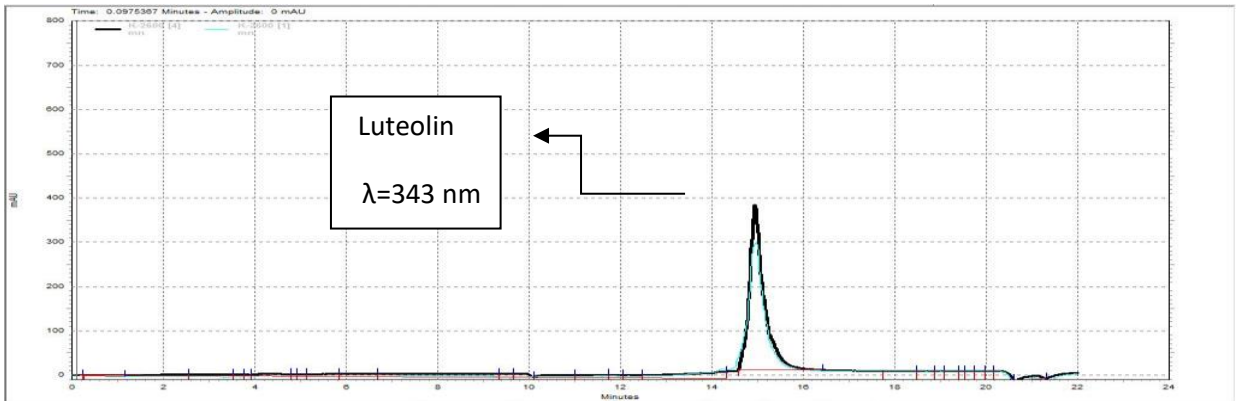
Strong antioxidant activity of plant extracts has a direct correlation with their high levels of phenols and flavonoids. Among the phenolic compounds, flavonoids are considered as the most potent antioxidant compounds. Quercetin and luteolin are two flavonoid compounds. These compounds have high antibacterial and antioxidant properties [22,23]. Investigating of the amount of these compounds in the C. arvence plant is very important due to its medicinal, nutritional and antioxidant properties. This investigation was performed for the first time. Obtained results of this study showed that both compounds are present in the extract of aerial organs of these plants. The presence of these compounds is consistent with the significant antioxidant activity of this extract. The results are listed in table 1. On the other hand, comparing the amounts of quercetin and luteolin in two populations A and B shows that the amounts of quercetin are (0.038, 0.113 mg/gr, respectively) and the amounts of luteolin are (0.233, 0.116 mg/gr, respectively). The amount of quercetin in B is more than Aand conversely for luteolin. Levels of luteolin and quercetin in sample (A, B respectively) can be due to the environmental and climatic conditions affecting the amount of metabolic compounds.

Considering the adverse effects of synthetic antioxidants on human health, further studies on the extraction, purification and application of C.arvence extract in the food industry are recommended. Identification and application of the active compounds of this medicinal plant can result in its better utilization.

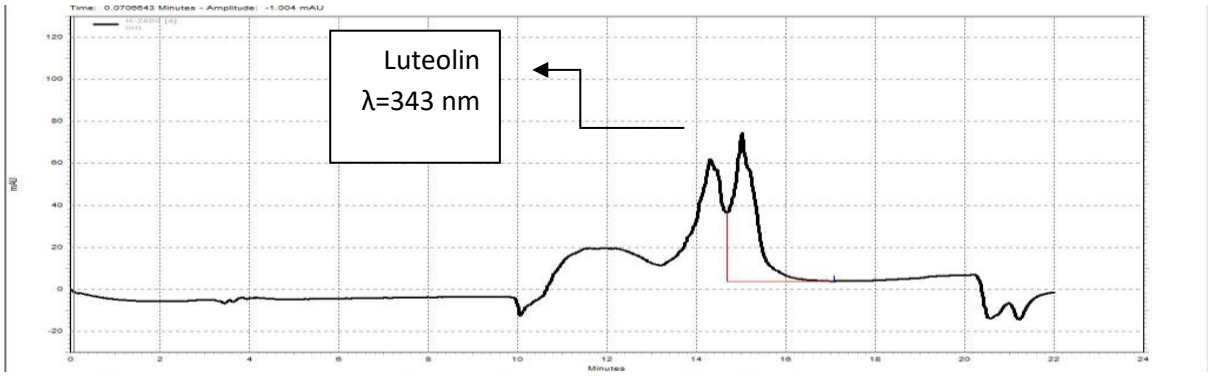
Table 1. The amount of Quercetin and Luteolin obtained from aerial organ (mg/g*)

	Quercetin	Luteolin
C.arvence (A)	0 .038	0.233
C.arvence (B)	0.113	0.116

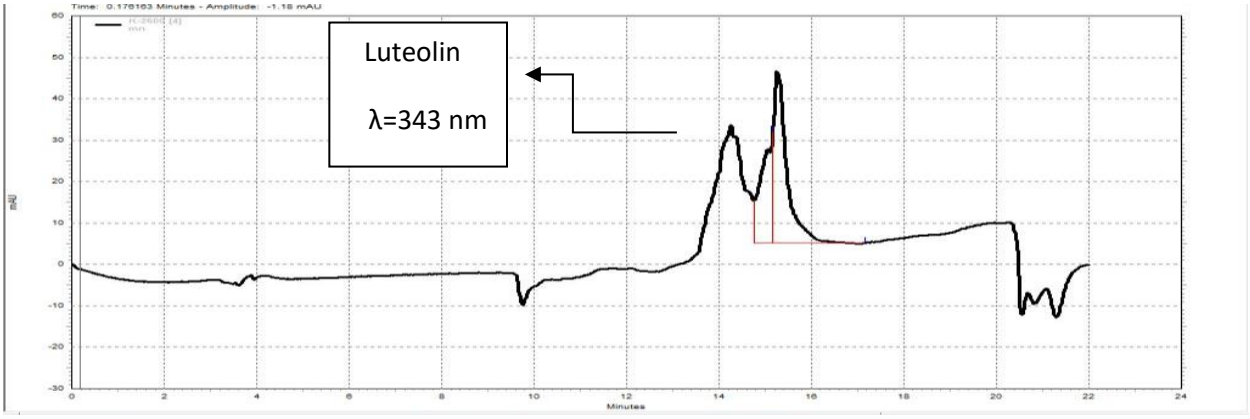
***** Dry weight sample



(a)The chromatogram of standard solution (0.1 mg/ ml) solution at 343 nm

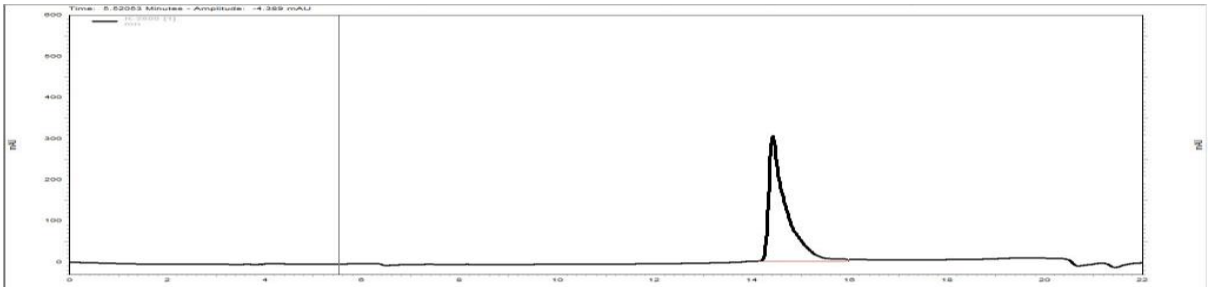


(b)The chromatogram of sample A solution at 343 nm

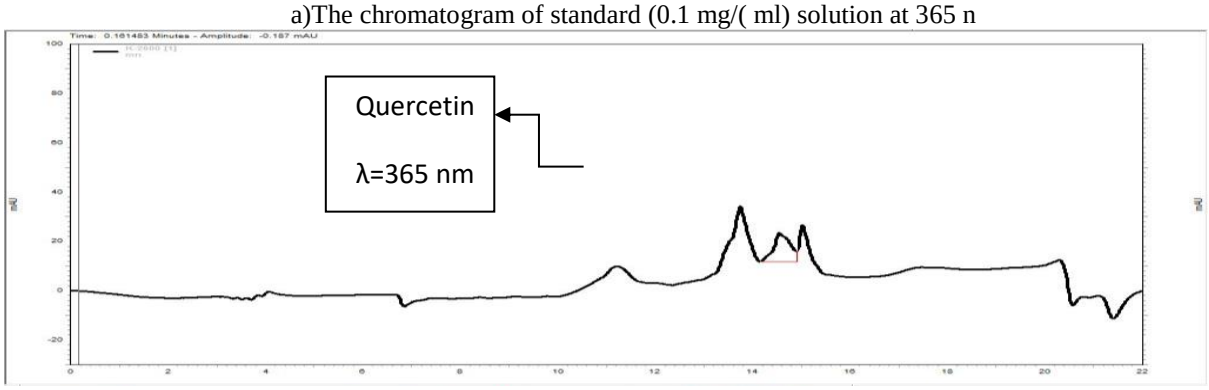


(c) The chromatogram of sample (B) solution at 343 nm

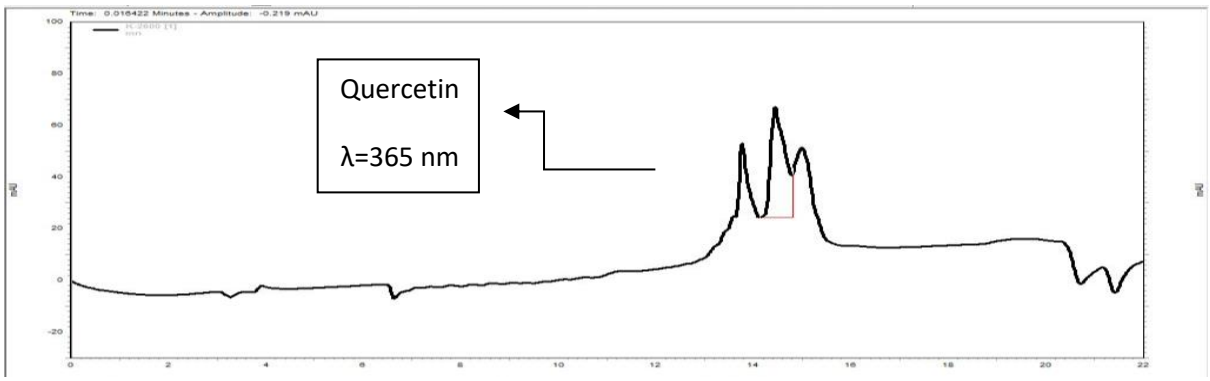
Figure 1. The chromatogram of luteolin



a)The chromatogram of standard (0.1 mg/(ml) solution at 365 n



(b)The chromatogram of sample(A) solution at 365 nm.



(c) The chromatogram of sample(B) solution at 365 nm.

Figure 2. The chromatogram of Quercetin

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