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Original Research Article

Quantitative HPLC analysis of two key flavonoids from *Scutellaria pinnatifida* A. Hamilt subsp *alpine* roots

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Abstract

Background: Flavonoids are natural phenolic compounds that are found in plants. Luteolin is a common flavonoid that exists in many types of plants and baicalin is one of the major flavone components of the genus *Scutellaria*. One of the Iranian species of *Scutellaria* is *Scutellaria pinnatifida* A. Hamilt. ssp. *alpina*.

Materials and Methods: In this study, total flavonoid content of the methanolic extract of roots was determined using $AlCl_3$ method and also the amount of baicalin and luteolin was evaluated by HPLC method.

Results: The amount of flavonoid in the methanolic extract was 91.3 mg quercetin/g of dry extract. The eluted baicalin was monitored at 290 nm from 4.6 to 4.7 min and for luteolin from 6.3 to 6.5 min. In roots of *S. pinnatifida*, the amount of baicalin and luteolin was 0.905% and 0.406%, respectively.

Conclusion: The present study showed that *S. pinnatifida*, similar to other species from the *Scutellaria* genus contains significant amounts of baicalin and luteolin.

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Introduction

Flavonoids are natural phenolic compounds found in different plants (Beecher, 2003). Their structure consists of two fused rings, an aromatic ring and an oxygen-containing heterocyclic ring that are attached by carbon–carbon bond to an aromatic ring. According to their chemical structure, flavonoids are classified as flavones, flavanones, flavonols, isoflavonoids and flavans (Beecher, 2003). Flavonoids show different biological activities such as antineoplastic (Li et al, 2007), antioxidant (Sang et al, 2005), antifungal (Friedman, 2007), and anti-inflammatory activities (Middleton, 1998). Luteolin (3', 4', 5, 7-tetrahydroxyflavone) is a hydroxylated flavone derivative, and similar to other flavonoids, it has a yellow crystalline appearance. It is a common flavonoid which exists in many types of plants (Figure 1).

Plants with high luteolin content have been used in Chinese traditional medicine for treating various diseases such as hypertension, inflammatory disorders, and cancer (Lin et al, 2008). Luteolin is one of the most cytotoxic flavonoids against HeLa cells (Mori et al, 1988). Protective effects of luteolin against development of diabetic nephropathy involved changes in superoxide dismutase activity, malondialdehyde content and expression of Heme Oxygenase-1 protein (Wang et al, 2011). Luteolin is found in *Scutellaria* genus (e.g. in *S. discolors* (Miyaichi et al, 1987), *S. barbata* (Wang, 1981), and *S. linearis* (Hussain et al, 2008)). Luteolin isolated from *S. barbata* showed antineoplastic activity and inhibited the proliferation of HL-60 (IC50= 18.4 µM) (Sonoda et al, 2004).

Baicalin (5,6,7-trihydroxyflavone-7-O-glucuronide) is one of the major flavone components of dried roots of *S. baicalensis* (Figure 1). Chinese Pharmacopoeia suggests that the content of baicalin in *S. radix* should be more than 9% (Yang et al, 2002). Baicalin is found in *S. baicalensis* (Tomimori et al, 1984), *S. amoena* (Xiao et al, 2003), *S. viscidula* (Zhang et al, 2005), *S. barbata* (Lin and Shieh, 1996) and *S. lateriflora* (Makino Shieh, 2008). Interestingly, baicalin could inhibit the proliferation of prostate cancer cells (Chan et al, 2000).

Scutellaria genus belongs to the family Lamiaceae and includes about 350 species commonly known as skullcaps (Willis, 1966). Almost 295 compounds have been obtained from 35 species of this gunes. Flavonoids and terpenoid compounds, which are phenolic chemicals, are the two main groups of constituents. Flavonoids and their derivatives are the main components of *Scutellaria* genus and the main flavonoids of

this genus are baicalin, baicalein and wogonin (Shanga et al, 2010).

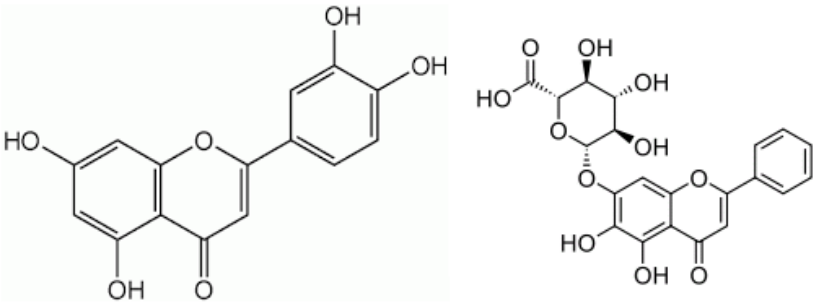

Figure 1. chemical structure of luteolin and baicalin.

According to the literature, 20 species of *Scutellaria* genus grow in Iran (Ghahreman and Attar, 1999) and 10 species and two hybrids are endemic to this country (Li et al, 2007). One of the Iranian species of *Scutellaria* is *S. pinnatifida* A. Hamilt. ssp. *alpina*. In this study, the amount of two key flavonoids namely, baicalin and luteolin was determined by HPLC method.

Materials and Methods

Chemicals and reagents

Methanol (Chromasolv, ≥ 99.9%, Sigma–Aldrich), Folin-Ciocalteu reagent (F9252, Sigma–Aldrich), Na_2CO_3 (451614, anhydrous powder, 99.999%, Sigma–Aldrich), aluminum chloride (563919, anhydrous powder, 99.999%, Sigma–Aldrich), quercetin (Q4951, ≥ 95%, Sigma–Aldrich), luteolin (L 9283, ≥ 95%, Sigma–Aldrich), and baicalin (572667, ≥95%, Sigma–Aldrich) were purchased.

Analytical instrumentation

The analytical HPLC system consisted of an LC-6AD pump (Shimadzu, Japan), an SPD-M20AUV detector (Shimadzu, Japan), and a VP-C_{18} analytical column (4.6 mm×150mm) (Shimadzu, Japan).

Preparation of the methanolic extract of *Scutellaria pinnatifida*

S. pinnatifida (100 g) was collected from Bojnurd, North Khorasan province, Iran, in June 2013. The plant was identified and a voucher specimen (No. 11868) was deposited at the Herbarium of Research Center of Natural Products and Medicinal plants, North Khorasan University of Medical Sciences,

Bojnurd, Iran. The roots of the plant were dried at room temperature, cut and macerated in methanol for 48 hr at room temperature. The whole extract was filtered and the solvent was evaporated under vacuum at 40˚C (Prachayasittikul et al, 2008).

Determination of total flavonoid content

Flavonoid quantification was done using aluminum chloride method. Here, 0.5 ml of extracts was mixed with 1.5 ml of methanol, 0.1 ml of potassium acetate (1 M), 0.1 ml of 10% aluminum chloride and 2.8 ml of distilled water. Then, the mixture was incubated for 30 min at room temperature and the absorbance of the samples was measured at 415 nm. All experiments were done in triplicates and the values were expressed in terms of flavonoid content (quercetin equivalent: QE per dry weight of extract). The calibration curve of quercetin 12.5–100 µg/mL in methanol was plotted (Chang et al, 2002).

HPLC analysis of luteolin and baicalin

Baicalin as a standard compound was dissolved in mobile phase yielding concentrations of 1-500 µg/ml. Also, the methanolic extract was prepared at the concentrations of 50 and 200 µg/ml. The solutions were filtered through a 0.45 µm membrane filter. The linearity of calibration curve was checked following HPLC analysis of baicalin and the methanolic extract. The eluted baicalin was monitored at 290 nm from 4.6 to 4.7 min and for luteolin, from 6.3 to 6.5 min. Concentrations of components were calculated according to peak heights. The mobile phase, methanol-H_2O-acetic acid (50: 45:5, V/V/V%), as well as the other chromatographic conditions, showed high performance in the separation of the flavonoid (Daigle and Conkerton, 1982). Evaluation of each point was repeated three times and each calibration curve was fitted.

The resolution between the peaks of the main flavonoids was determined by analysing the chromatograms obtained from standard solution and the sample solution.

Results

In the present study, the extraction yield of the methanol solvent was 10.10%. Total flavonoid contents were determined using the following equation:

$$Y = 0.004\,X + 0.006\ (R^2 = 0.996)$$

Moreover, the amount of flavonoid in the methanolic extract was 91.3 mg quercetin/g of dry extract. HPLC chromatograms of baicalin and luteolin as standards were shown in Figure 2.

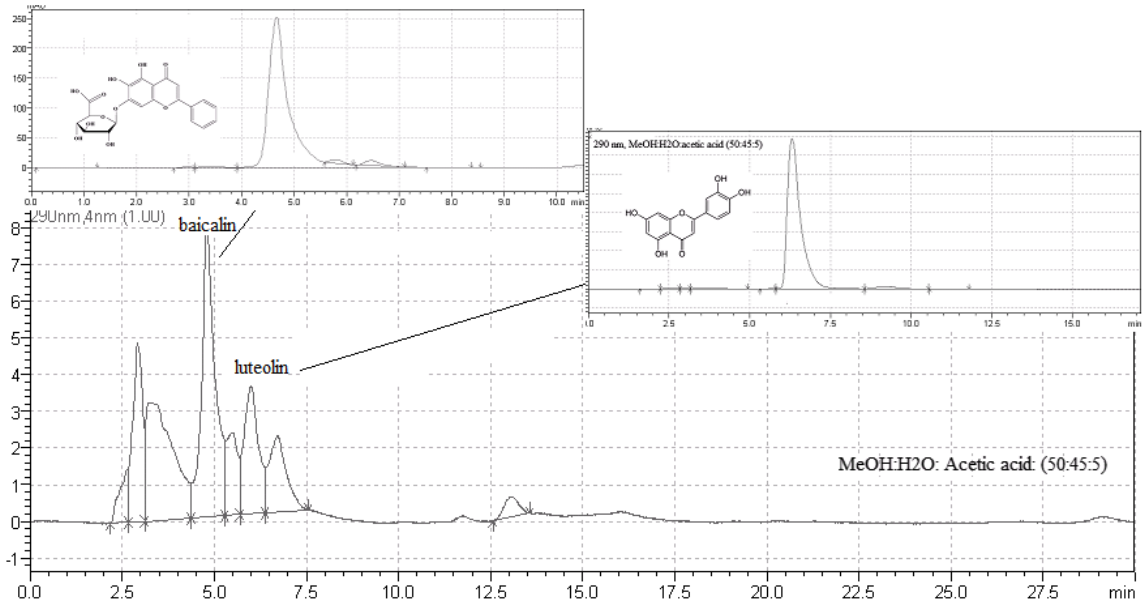

Figure 2. HPLC Chromatograms of baicalin, luteolin and methanolic extract of *S. pinnatifida* (the mobile phase was methanol-H_2O-acetic acid (50: 45: 5 %V/V/V)).

Luteolin and baicalin were well separated, and the retention time for luteolin and baicalin was about 4.6 and 6.3 min, respectively.

Standard curves plotted for luteolin and baicalin are shown in Figure 3.

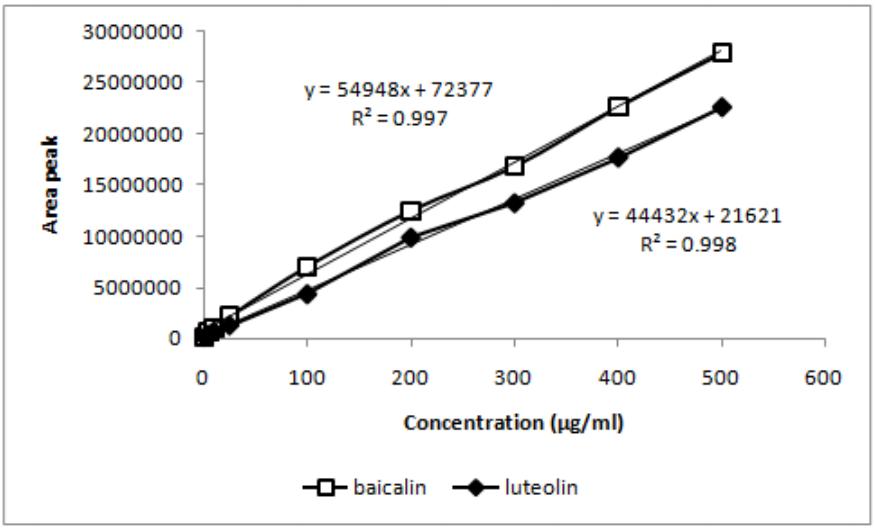

Figure 3. Standard curve of luteolin and baicalin

The linear equation was Y = 54948X+72377, (R^2= 0.997) for baicalin and Y= 44432 X+21621 (R^2= 0.998) for luteolin. X indicates the concentration of baicalin and luteolin and Y indicates the peak area of baicalin and luteolin. HPLC chromatogram of the methanolic extract is shown in Figure 2. The quantities of luteolin and baicalin were determined based on their peak area (Tables 1 and 2).

Discussion

Flavonoid compounds are important chemicals with antioxidant activity. Our study showed that baicalin and luteolin were the two important flavonoids in *S. pinnatifida*. In our study, for determination of baicalin and luteolin in *S. pinnatifida*, solvent system consisted of methanol-H_2O-acetic acid (50: 45:5, %V/V/V), that provided optimum separation of luteolin and baicalin from the plant extract. The specificity

of the method was evaluated by analysis of blank, standard and sample solution chromatograms (Figure 2). According to HPLC chromatograms, the peak that appeared at 4.6 min was related to baicalin and the other peak appeared at 6.3 min was related to luteolin. Linearity was evaluated by the correlation coefficient R^2, and all R^2 values for compounds were greater than 0.99 (examined standard concentration range was from 1 to 500µg/ml). The quantitative method developed here was successfully in simultaneous analysis of two different compounds in the extracts of *S. pinnatifida.*

Conclusion

The present study showed that *S. pinnatifida* such as other species from the *Scutellaria* genus (e.g. as *S. radix*) contains significant amounts of baicalin and luteolin.

Acknowledgements

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Conflict of interest

The authors have no competing interests to declare.

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