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Evaluation of antioxidant activity of Luteolin, isolated from *Dracocephalum kotschyi*

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Abstract

Background: *Dracocephalum kotschyi* is a plant from the family Labiatae. This family includes over 60 species, from which, 8 species are endemic to Iran. One of these species, is *D. kotschyi* and has been used as an anti-fever, anti-rheumatism, and anti-inflammatory herb in Iranian traditional medicine. The aim of this study was extraction of luteolin as a pharmacologically active flavonoid from *D. kotschyi* and evaluation of its antioxidant properties.
Materials and Methods: Methanolic extract of the plant was prepared by maceration method and then, using chromatography methods (i.e. column chromatography and thin layer chromatography), luteolin was extracted and the ferric reducing power of it was evaluated. Ascorbic acid (Vit C), Butylated hydroxytoluene (BHT), and quercetin were used as standards.
Results: According to our results, the ferric reducing potential of luteolin was more than that of BHT and less than that of other standards.
Conclusion: *D. kotschyi* could be introduced as a source of luteolin in medical sciences.

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Introduction

Chromatography methods are being used for extraction of naturally occurring compounds and HPLC is one of the mostly used methods for purification of compounds (Marston, 2007). *Dracocephalum* belongs to the family Labiatae and includes over 60 species from which, eight species are found in the flora of Iran (Rechinger, 1986; Mozaffarian, 1996). This genus has been used in Iranian traditional medicine for stomach and liver disorders, headache and congestion (Mirheydar, 1995). *Dracocephalum kotschyi*, one of the three endemic species growing in North Khorasan province, Iran (Rechinger, 1986; Mozaffarian, 1996), has several medicinal properties including antihyperlipidemic, immunomodulatory, antinociceptive and cytotoxic effects (Sajjadi et al, 1998; Amirghofran et al, 2000; Golshani et al, 2004; Jahanian et al, 2005). Flavonoids such as calycopterin, xanthomicrol, isokaempferide, luteolin and apigenin have been isolated from *D. subcapitatum* (Saeidnia et al, 2005). It was shown that phenolic compounds such as flavonoids and rosmarinic acid in *D. moldavica* were responsible for antioxidant activity (Fattahi et al, 2013; Gohari et al, 2007). *D. kotschyi* is a rich source of natural antioxidants and flavonoids such as luteolin, apigenin, cirsimaritin, penduletin, xanthomicrol, calycopterin and rosmarinic acid (Prachayasittikul et al, 2008). Methoxylated flavones isolated from *D. kotschyi* have been reported to have anticancer effects (Jahanian et al, 2005; Rios et al, 1998).

The FRAP assay (ferric reducing ability of plasma) is a method for evaluation of the antioxidant activity of chemicals (Szollosi and Varga, 2002). FRAP assay depends on the reduction of ferric tripyridyltriazine complex to ferrous tripyridyltriazine. Ferrous tripyridyltriazine has an intensive blue color and is monitored at 593 nm (Benzie and Strain, 1996). FRAP method is sensitive in the measurement of total antioxidant power of the plant homogenates and pharmacologically active compounds of the plants (Szollosi and Varga, 2002).

The target of this study was extraction of luteolin from the methanolic extract of *D. kotschyi* and evaluation of its antioxidant activity by FRAP assay.

Materials and Methods

Plant material

Plants material was collected from North Khorasan Province of Iran in June 2013. The plant was identified by an expert and a voucher specimen (voucher No. 36-1-2) was kept at the Natural Products and Medicinal Plants Research Center, North Khorasan University of Medical Sciences, Bojnurd, Iran.

Preparation of the methanolic extract

The aerial parts of the plant were dried in the shade and then cut into small pieces. Air-dried plant (100 g) was ground into powder and macerated in methanol at room temperature for 48 hr. After filtration, the extract was concentrated under vacuum at 40 °C (Bors et al, 1994).

Isolation of fractions from the methanolic extract

Concentrated methanolic extract (4 g) was subjected to column chromatography (60 × 2 cm; silica gel 60 A, 200–425 mesh), and the column was eluted with a mixture of petroleum ether-ethyl acetate, followed by a mixture of ethyl acetate-methanol in order to increase the polarity to obtain different fractions (F_1-F_{10}). Each fraction was subjected to thin-layer chromatography, using a mixture of chloroform: methanol (10:1 V/V). Then, flavonoids were visualized under ultraviolet (UV) light. The fractions with a similar retention factor (Rf) were combined resulting in 5 fractions. F_4 was obtained with the elution of 40-100% ethyl acetate in petroleum ether and it was pure when it was examined by TLC because only one spot was observed under UV light. Afterwards, it was injected to high performance liquid chromatography (HPLC). The analytical HPLC system consisted of an LC-6AD pump (Shimadzu, Japan), an SPD-M20AUV detector (Shimadzu, Japan) and a VP-C18 analytical column (4.6 mm×150 mm, Shimadzau, Japan) and the wave length was set at 280 nm. The fraction F4 (955 mg) and standard compound luteolin were eluted by solvents MeOH/ H_2O 90:10 (V/V %) (pH 3 by adding acetic acid) (Daigle and Conkerton, 1986) and both of them had the same retention times; thus, the fraction F4 was luteolin (HPLC chromatogram of F4 and luteolin were shown in Figure 1).

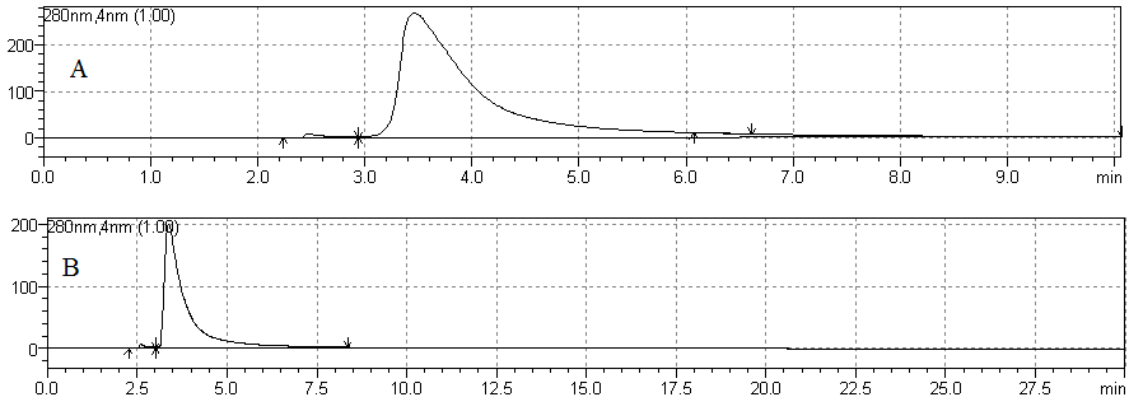

Figure 1. HPLC chromatograms of F_4 and luteolin.

Ferric reducing antioxidant power (FRAP) assay

The antioxidant potential of the methanolic extract and the fraction F_4 was measured using ferric reducing antioxidant power (FRAP) assay. The FRAP reagent contained 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, 20 mM $FeCl_3.6H_2O$ and 0.3 M acetate buffer (pH 3.6). Here, 3 ml of freshly-prepared FRAP reagent was mixed with 100 µl of each sample and incubated at 37 ^oC for 10 min in a water bath. After the incubation period, the absorbance was measured at 593 nm. Aqueous solutions within the concentration range of 0-1 mM ($FeSO_4.7H_2O$) were used for calibration. FRAP values were expressed as mean ± standard error (SE) mmol Fe (II) per gram. In this test, Vit C, BHT and quercetin were used as positive controls (Benzie and Strain, 1996).

Results

The yield of the methanolic extract was 11.11%. F_4 contained a pure compound and appeared as a yellow powder. When it was studied with TLC under UV-light, it was pure and it had the same spot as luteolin in TLC. Also, they had similar retention times as assessed by HPLC; so, F_4 was luteolin (its structure was shown in Figure 2).

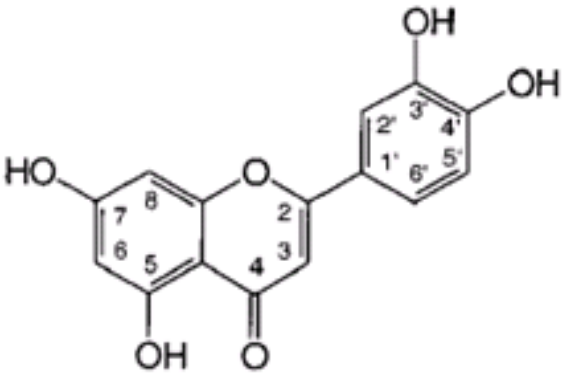

Figure 2. Chemical structure of luteolin.

From 100 gram of dried plant, 955 mg luteolin was isolated. The equation of FRAP for standard solution was: Y= 0.435X+ 0.075 (R^2 =0.988). The antioxidant activity of each sample was calculated and presented in Table 1 and Figure 3.

Table 1. Ferric reducing antioxidant power of the methanolic extract of *D. kotschyi* and luteolin.

sample	FRAP value (mmol Fe^{2+}/g dried sample)
Methanolic extract	32.357 ± 0.9
BHT	14.3± 0.46
Ascorbic acid	81.6± 0.09
Quercetin	37.2± 1.59
Luteolin	32.4± 0.54

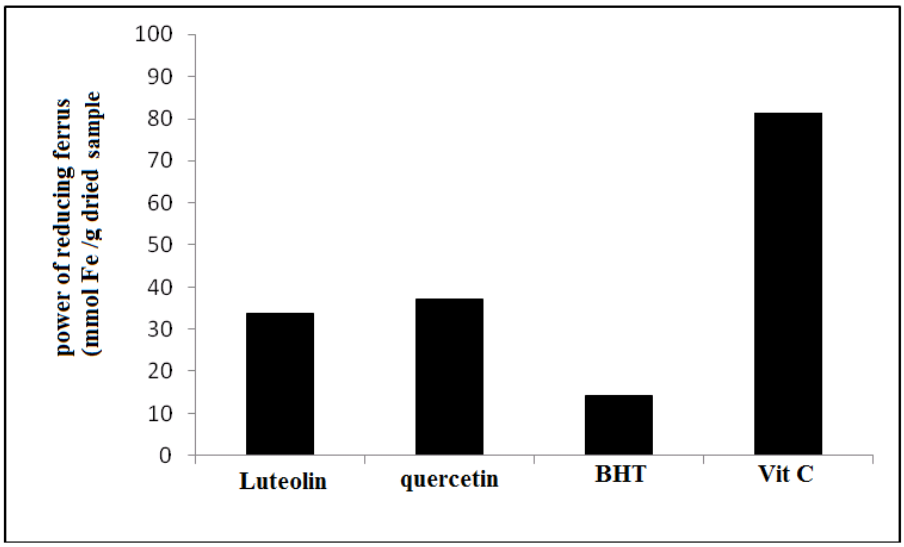

Figure 3. Ferric reducing antioxidant power of luteolin and the standards.

Discussion

As shown in Figure 3, based on the data from FRAP assay, antioxidant potential of luteolin was higher than BHT, but it was lower than Vit C and quercetin. Molecular weight, number of aromatic rings and hydroxyl group positions affect the antioxidant activity of compounds (Zhang et al, 2011). Flavonoids with a hydroxyl group in their structure have antioxidant properties as they donate hydrogen to a free radical (Miliauskas et al, 2004). Plants rich in luteolin have been used in Chinese traditional medicine for treating various diseases such as hypertension, inflammatory disorders, and cancer (Bors et al, 1994). Luteolin has been shown to have antioxidant (Bors et al, 1994; Shimoi et al, 1994), anti-tumorigenic (Yasukawa et al, 1989), anti-inflammatory (Yamamoto et al, 1998) and anti-allergic activities (Pettit et al, 1996). In previous studies, luteolin was introduced as a treatment for multiple sclerosis (MS) (Theoharides, 2009) and *D. kotschyi* from North khorasan province, Iran which possesses high amounts of this compound can be used as a medical plant for treatment of MS.

Conclusion

In conclusion, our findings suggested that *D. kotschyi* could be introduced as a source of luteolin in medical sciences. Further studies are required to investigate pharmacological activities of the active compounds of this plant.

Acknowledgements

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Conflict of interest

The authors have no competing interests to declare.

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