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Research Article

New sources of arbutin: *Onobrychis nitida* and *Onobrychis galegifolia*

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Abstract

Arbutin (4-Hydroxyphenyl-*β-D*-glucopyranoside) is a mono glycosidic form of hydroquinone and has been widely used in cosmetics for a long time due to skin whitening and freckle removing properties. In this study, arbutin was isolated from the methanol extract of *Onobrychis galegifolia* using flash chromatography. The structure of arbutin was elucidated by ^{1}H and ^{13}C -NMR spectroscopy and as well as 2D NMR techniques. In addition, quantitative analysis of arbutin found in *O. galegifolia* and *O. nitida* species was done using HPLC-DAD. According to the results, the arbutin content of methanol extracts of *O. galegifolia* and *O. nitida* was found 212.02 and 196.46 mg/g extract respectively. In addition, the arbutin content of cold-water extracts of *O. galegifolia* and *O. nitida* was found 128.2 and 99.40 mg/g extract. The amount of arbutin in dry weights was calculated for about 5.66% to 6.86%.

Keywords: Arbutin, *Onobrychis nitida*, *Onobrychis galegifolia*

Arbutin'in yeni kaynakları: *Onobrychis nitida* and *Onobrychis galegifolia*

Öz

Arbutin (4-Hidroksifenil-*β-D*-glukopiranozit), hidrokinonun mono glikozidik formudur ve cilt beyazlatma ve çil giderme özellikleri nedeniyle kozmetiklerde uzun süredir yaygın olarak kullanılmaktadır. Bu çalışmada; arbutin flash kromatografi kullanılarak *Onobrychis galegifolia*'nın metanol ekstraktından izole edilmiştir. Arbutin yapısı, ^{1}H ve ^{13}C -NMR spektroskopisi ve ayrıca 2D NMR teknikleri ile aydınlatıldı. Ek olarak, *O. galegifolia* ve *O. nitida* türlerinde bulunan arbutinin kantitatif analizi HPLC-DAD kullanılarak yapıldı. Sonuçlara göre, *O. galegifolia* ve *O. nitida*'nın metanol ekstraktlarının arbutin içeriği sırasıyla 212.02 ve 196.46 mg/g ekstrakt olarak bulundu. Ayrıca, *O. galegifolia* ve *O. nitida*'nın soğuk su ekstraktlarının arbutin içeriği 128.2 ve 99.40 mg/g ekstrakt olarak bulundu. Kuru ağırlıklardaki arbutin miktarı, yaklaşık% 5.66 ila% 6.86 arasında hesaplandı.

Anahtar Kelimeler: Arbutin, *Onobrychis nitida*, *Onobrychis galegifolia*

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1. Introduction

The *Onobrychis* genus comprises a few agro-economically important forage legume species and some *Onobrychis* species are cultivated in many parts of the world because of their flavor and drought resistance as a portion of animal food. Recent studies show that it has highly beneficial properties for animals because of its tannin and polyphenolic composition. The phenolic contents of sainfoins are assumed to contribute to its nutritive value and bioactive properties The *Onobrychis* species (sainfoins) known as “korunga” in Turkey. *Onobrychis nitida*, which is an endemic species to Turkey, and *Onobrychis* galegifolia also known as “Fırat korungası” and “darp korungası”, respectively. The objectives of the current study were, to determine the arbutin contents of two sainfoin species (*O. nitida* and *O. galegifolia*) growing in the flora of Erzincan to contribute to knowledge about chemical content these two species.

2. Material and Methods

2.1. Plant materials

Plant materials was collected İliç-Kemah Road 32th km in June 2018 from jipsy rocks, Erzincan. Plant materials were authenticated by Prof. Dr. Ali Kandemir, a voucher specimen was deposited in Erzincan Binali Yıldırım University Herbarium.

2.2. General experimental process

^{1}H, ^{13}C, DEPT, HETCOR and COSY NMR spectra were recorded on a Bruker Avance II 400 MHz spectrometer in DMSO-d6. Analytical HPLC analysis was carried out on a Thermo Scientific Ultimate 3000 HPLC with Agilent Zorbax C18 column (4.6X250 mm, 5 µm). The arbutin was detected at 290 nm with a linear gradient from 5 to 55% ACN in 0.5 % phosphoric acid in water for 15 min. The column temperature was set at 35 °C. Flash chromatography separation of arbutin was carried out using a Buchi Reveleris X2 instrument with an Ecoflex silica gel packed column (40 g, 50 µm).

2.3. Isolation of Arbutin

The crude methanol extract (8.0 g) was dissolved in 30 mL of water and non-soluble parts were removed. An aliquot of 10 mL of this solution was injected into the reverse phase column (30x150 mm). 250 mL of following mixtures of water and methanol were passed column: 100:0 (F-1), 90:10 (F-2), 80:20 (F-3), 70:30 (F-4), 60:40 (F-5), 50:50 (F-6) and 40:60 (F-7). The F-1 and F-2 combined according to TLC basis (See Figure 1) then concentrated under vacuum to give an arbutin-rich mixture (3 g). The mixture was loaded onto to flash chromatography system and the extract was eluted with a linear gradient from 100:0 (A: B) to 80:20 A: B for 30 min at a flow rate of 20 mL/min. Here, A was a mixture of EtOAc: water: acetic acid (7:1.5:0.5) and B was methanol. The automatically collected fractions (18-21 min) based on the signals of ELSD were pooled, concentrated to the dryness (See Figure 1). Arbutin was obtained as white crystalline (600 mg).

2.4. Quantification of arbutin

Ten mg of arbutin accurately weighed and solved in 10 mL of deionized water to get a 1000 ppm stock solution. Five concentrations were prepared to contain 500, 250, 125, 62.5 and 31.25 ppm by serial dilution. After analyzing these concentrations, a calibration curve was obtained with an R^2=0.9999 value and y=0.0577x+0.095 equation. LOQ and LOD were calculated as 0.83 and 0.33 ppm, respectively. The extraction procedure was as

follows: 4 g of well-grounded aerial parts of plant materials were extracted in 100 mL of methanol in the ultrasonic bath for 30 min in ambient temperature. The solvent was removed by filtration and fresh 100 ml of methanol was added. The process was repeated triple. The same extraction process was repeated for cold-water extracts. The solvents were evaporated to the dryness then a 20 mg/mL stock solution was prepared and filtrated using 0.22 µm syringe filter. The methanol and cold-water extracts were diluted 1:20 and 1:10 respectively. The final solutions were injected into HPLC. The arbutin contents of extract were expressed as mg arbutin/g extract and % arbutin content of dry biomass. The HPLC-UV chromatograms of standard arbutin and plant extracts were given in Figure 2.

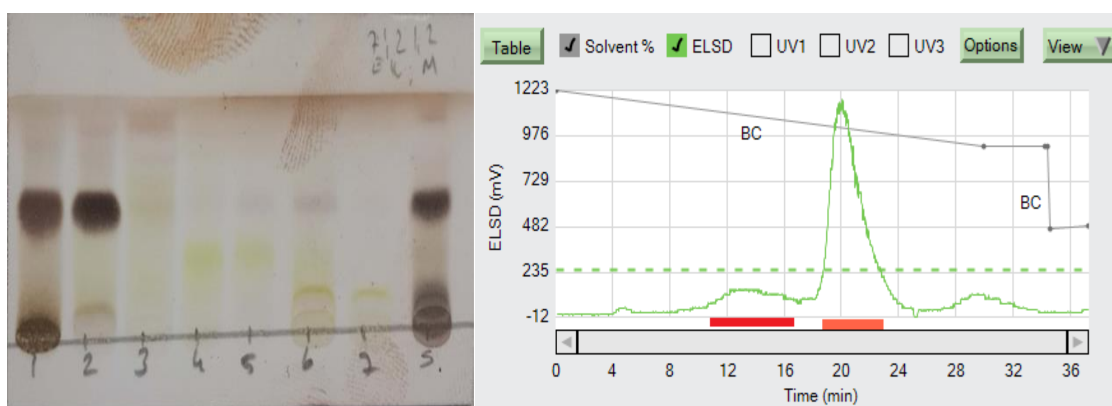

Figure 1. TLC view and Flash chromatography chromatogram of C18 fraction F-1 and F-2

3. Results

3.1. NMR analysis:

The ^{1}H spectra of arbutin were exhibited two doublets with *J*=8.86 Hz at 6.67 and 6.86 ppm, which approval an AB system. Aliphatic protons at 2.95-3.47 ppm and a doublet at 4.65 (d, *J*=7.50 Hz) show a β-glycosidic moiety. The correlation 4.65 with 152.7 noticed from the HMBC spectrum established a the glycosidic bond is between C4 and C1′.

^{1}H NMR, (400 MHz, in DMSO-d6), δ_H 6.67 (d, 8.86 Hz, 2H, H2 and H6), 6.87 (d, 8.86 Hz, 2H, H3 and H5), 4.65 (d, 7.50 Hz, 1H, H1′), 2.93-2.97 (m, 1H, H2′), 3.09-3.13 (m, 1H, H3′), 3.02-3.06 (m, 1H, H4′), 3.13-3.17 (m, 1H, H5′), 3.67-3.72 (m, 1H, H6′a), 3.43-3.47 (m, 1H, H6′b). ^{13}C NMR (100 MHz, in DMSO-d6) δ_C 150.8, (C1), 115.9 (C2 and C6), 118.2 (C3 and C5), 152.7 (C4), 102.2 (C1′), 73.8 (C2′), 77.1 (C3′), 70.3 (C4′),77.4 (C5′), 61.3 (C6′). The ^{1}H and ^{13}C NMR spectra were identical to those of arbutin (See Figure 2).

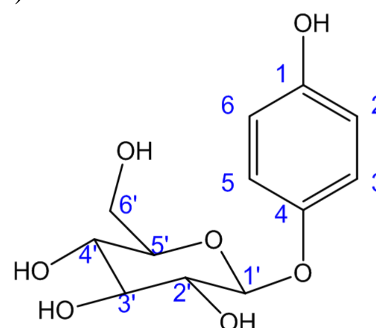

Figure 2. Chemical structure of arbutin

3.2. Quantitive analysis

The results were given in Table 1. HPLC analysis showed that arbutin could be extracted effectively with methanol than cold water for two analyzed plant material. *O. galegifolia* calculated as % arbutin content of dry biomass as follows %6.86 and %5.66 for *O. galegifolia* and *O. nitida*, respectively. Arbutin is found in various plants such as *Arctostaphylos uvaursi*, *Vaccinium vitis-idea*, *Pyrus communis.* and some *Origanum* species. *Bergenia crassifolia* is one of richest arbutin has been found richer than *O. nitida* in the aspect of arbutin. The arbutin contents of plants were

Table 1. Quantitatively calculated arbutin content of *O. nitida and O. galegifolia*

	Extraction solvent	mg/gr extract	% Dry biomass
Onobrichys galegifolia	Cold water	128.22±1.44	6.86
	MeOH	212.02±0.98	
Onobrichys nitida	Cold water	99.41±1.22	5.66
	MeOH	196.46±2.01	

containing plant (~%20 of dry weight). According to our findings *O. nitida* and *O. galegifolia* could be considered as new sources of arbutin by %5.66-6.86 arbutin content of dry weights, respectively.

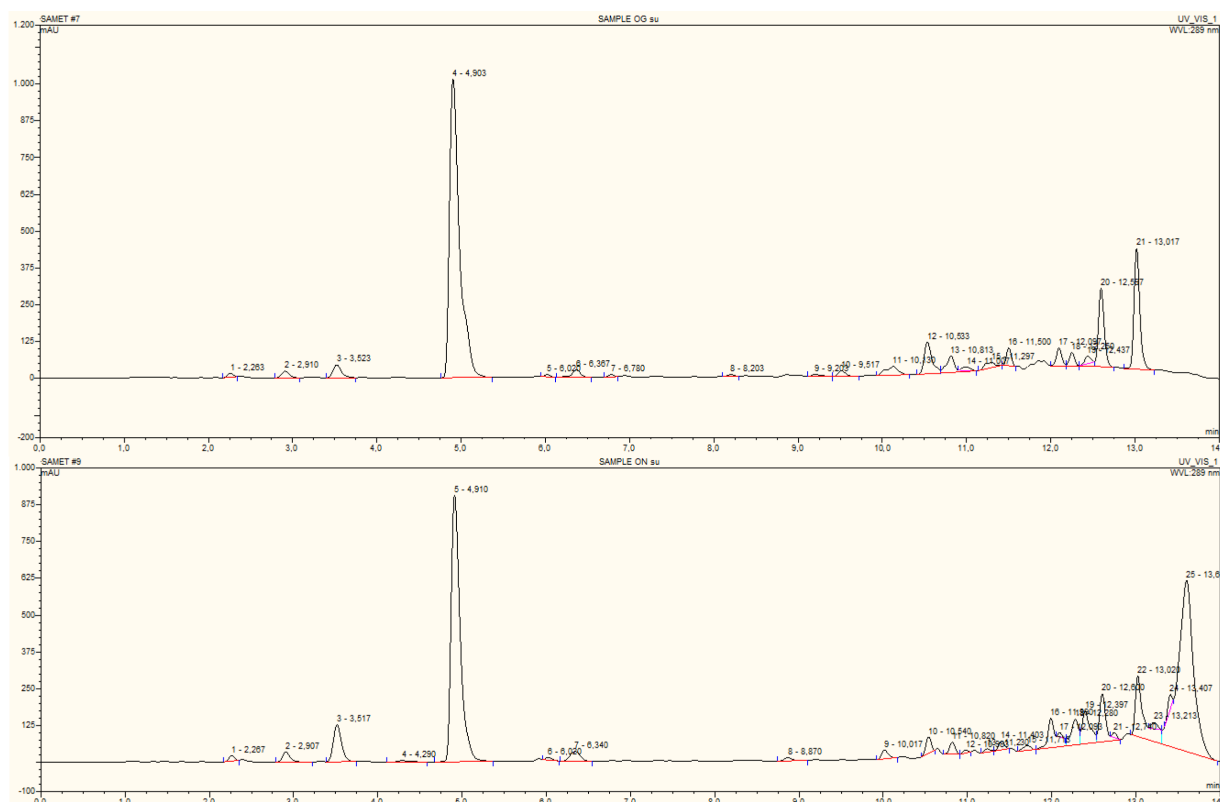

Figure 3. HPLC chromatograms of cold-water extracts of *Onobrychis galegifolia* and *Onobrychis nitida* (290 nm)

4. Conclusions

Arbutin and polyphenolic content of some *Onobrychis* species were reported previously (Moniava 1970, Bol', Kompantsev et al. 1976, Luk'yanchikov 1982, Marais, Mueller-Harvey et al. 2000, Regos, Urbanella et al. 2009, Regos and Treutter 2010). *Onobrychis nitida* plant is an endemic species to Turkey flora (Kandemir 2009). Although some research has been carried out on HPLC analysis of chemical content of *O. nitida* (Kandemir, Türkmen et al. 2018) no single study exists which reports both occurrence and quantitative analysis of arbutin in *O. nitida*.

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