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Source / Izvornik: **Acta Pharmaceutica, 2006, 56, 245 - 250**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:163:801777

Acta Pharm. 56 (2006) 245–250 Short communication

Qualitative and quantitative analysis of flavonoids of the strawberry tree – *Arbutus unedo* L. (*Ericaceae*)*

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The leaves and fruits of strawberry tree – *Arbutus unedo* L., collected from two separate geographic locations in Croatia were investigated to determine their flavonoid composition and content.

Quercitrin, isoquercitrin, hyperoside and rutin were identified in all leaf samples by means of thin-layer chromatography; the fruits contained only isoquercitrin. Chlorogenic acid was present in some leaf samples. The content of flavonoids depended on the plant organ investigated, date of collection and the locality. Spectrophotometric determination of the flavonoids indicated that the leaves are richer in flavonoids (0.52–2.00%) than fruits (0.10–0.29%).

Keywords: *Arbutus unedo* L. (*Ericaceae*), flavonoids, TLC, spectrophotometry

Accepted November 30, 2005

Strawberry tree, *Arbutus unedo* L. (*Ericaceae*), is an evergreen shrub or small tree reaching up to 12 m in height. It makes a very attractive, picturesque crown on a short single, or often, on multiple trunks. The species is widespread throughout the Mediterranean region, but rare in some parts of North Africa, growing in maquis, evergreen scrubs, woodland margins and on rocky slopes (1–3). Its leaves are alternate, simple, oblanceolate, dark green, leathery, short-stalked and toothed. The flowers are bell-shaped, with recurved lobes, 8–9 mm long, white, often tinged with pink or green, and honey-scented. The fruits are globose berries about 15–20 mm in diameter, ripening from yellow to scarlet and deep crimson. Since the fruits take about 12 months to ripen, the tree carries mature fruits and flowers at the same time, and the appearance of both during the winter months makes this plant very popular also for specimen plantings (1–3).

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* This work was presented at the Third Croatian Congress on Pharmacy with International Participation, Cavtat, April 27–30, 2005

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The leaves of *A. unedo* are used as a urinary antiseptic, against diabetes, an antidiarrheal, adstringent, depurative, antioxidant and as an antihypertensive (4–7). Chemical investigations of leaves and fruits indicated the presence of the essential oil, flavonoids, proanthocyanidins, iridoid glucosides, sugars, non-volatile and phenolic acids, and -tocopherol (4, 8–11).

The paper investigates the composition of flavonoids in the leaves and fruits of strawberry tree, collected from two locations in Croatia.

EXPERIMENTAL

Plant material and chemicals

The leaves and fruits of *A. unedo* were collected at two separate geographic localities in Croatia (the island of Dugi otok, Božava – 44° 08′ 30′′ N, 14° 54′ 30′′ E; the Pelješac Peninsula, Orebić – 42° 59′ 00′′ N, 17° 11′ 00′′ E). Samples of leaves were collected every month in 2003, while samples of fruits were collected in December 2003. Voucher specimens (No. 99450–99475) were deposited at the Department of Pharmaceutical Botany, Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia. Growing sites and collection dates are presented in Table I.

All solvents used were of analytical grade (Merck, Germany). Standards (quercetin, caffeic acid, quercitrin, isoquercitrin, hyperoside, chlorogenic acid and rutin) were purchased from C. Roth (Germany).

Air-dried, powdered leaves and fruits of *A. unedo* (1.0 g) were refluxed with 10.0 mL methanol for 30 minutes, filtered, and the filtrate was concentrated under reduced pressure and then the residue was taken up in 5.0 mL methanol (12).

Flavonoid and phenolic acid standards were prepared as 0.05% solutions in methanol.

Qualitative analysis

Thin-layer chromatography (TLC) was performed on precoated 20 x 20 cm TLC plates coated with 0.25 mm layers of silica gel 60 F_{254} (Merck). After application of the extract and standard solutions (10 L), the plates were developed for 19 cm in paper-lined all--glass chambers (Desaga, Germany) previously left to equilibrate for at least 30 min. Two chromatography solvents were used: ethyl acetate/formic acid/acetic acid/water, 100:11:11:26 (*V/V*) and ethyl acetate/formic acid/water, 8:1:1 (*V/V*) (12, 13). Visualization of the flavonoids and phenolic acids was achieved by spraying the sheets with natural products--polyethylene glycol reagent (NP/PEG)(Fluka Chemie, Switzerland). Typical intense fluorescence in UV light at = 365 nm was produced immediately on spraying (flavonoids appeared as orange-yellow bands, whereas phenolic acids formed blue fluorescent zones). Addition of polyethylene glycol solution lowered the detection limit and intensified fluorescence. The detection limit for the flavonoids and phenolic acids was reported as 2.5 g (12).

Quantitative analysis

The measurements were carried out with a spectrophotometer UV-1601 (Shimadzu, Japan).

The content of flavonoids calculated as quercetin in the plant samples was determined by the method of Christ and Müller (14). After acid hydrolysis (with 25% hydrochloric acid in acetone for 30 minutes at 100 °C), the liberated aglycones were spectrometrically determined at 425 nm by forming a complex with $AlCl_3$ in a methanol/ethyl acetate/acetic acid medium (15, 16).

Statistical analysis

The content of flavonoids was evaluated upon five independent analyses and data were expressed as means ± SD. The significance of a between-group difference was determined by Student's *t*-test using the SigmaStat (version 2.0, Jandel Corporation) program (16).

RESULTS AND DISCUSSION

TLC separation of flavonoids and phenolic acids from methanolic extracts of the *A. unedo* leaves indicated the presence of quercitrin (R_F = 0.86), isoquercitrin (R_F = 0.73), hyperoside (R_F = 0.69), and rutin (R_F = 0.47) in all samples. Chlorogenic acid (R_F = 0.50) was identified only in samples collected in Božava (Fig. 1). Both mobile phases enabled similar separation of investigated compounds.

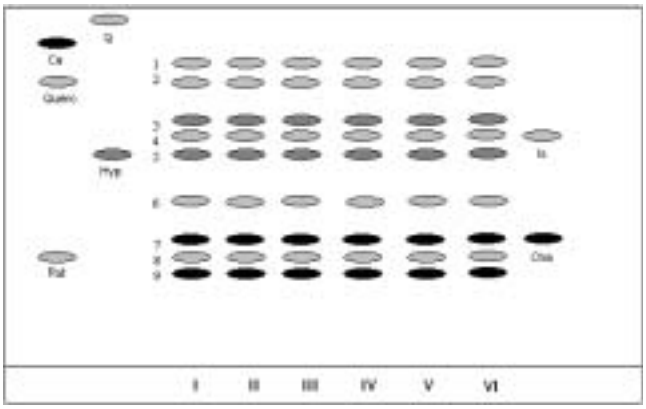

Fig. 1. Schematic TLC separation on silica gel, with ethyl acetate/formic acid/acetic acid/water (100: 11:11:26, *V/V*) as the mobile phase, of flavonoids and phenolic acids (1–9) in the leaves of *A. unedo* collected from January to June (lanes I–VI) at the locality Božava.
Key to the spots: Ca – caffeic acid, Querc – quercitrin, Rut – rutin, Q – quercetin, Hyp – hyperoside, Is – isoquercitrin, Cha – chlorogenic acid.

Table I. Dried basis content of flavonoids in the leaves and fruits of Arbutus unedo *L. (%)*[a]

Plant part	Collection date	Collection site	
		Božava	Orebić
Leaves	15. 1. 2003	1.33 ± 0.01[b]	0.59 ± 0.03[b]
	15. 2. 2003	1.54 ± 0.04 [b]	0.67 ± 0.02 [b]
	15. 3. 2003	1.29 ± 0.04 [b]	0.55 ± 0.01 [b]
	15. 4. 2003	1.34 ± 0.01 [b]	0.73 ± 0.01 [b]
	15. 5. 2003	1.41 ± 0.04 [b]	0.52 ± 0.03 [b]
	15. 6. 2003	1.30 ± 0.04 [b]	0.87 ± 0.01 [b]
	15. 7. 2003	2.00 ± 0.04 [b]	0.59 ± 0.03 [b]
	15. 8. 2003	1.59 ± 0.06 [b]	0.68 ± 0.03 [b]
	15. 9. 2003	1.45 ± 0.05 [b]	0.79 ± 0.05 [b]
	15. 10. 2003	1.40 ± 0.02 [b]	0.76 ± 0.02 [b]
	15. 11. 2003	1.36 ± 0.04 [b]	0.74 ±0 .01 [b]
	15. 12. 2003	1.70 ± 0.06 [b]	0.80 ± 0.02 [b]
Fruits	15. 12. 2003	0.29 ± 0.03 [b]	0.10 ± 0.02 [b]

[a] Mean ± SD, $n = 5$.
[b] Statistically significant difference ($p < 0.001$) between two sites on the same collection date.

Samples of fruits from both localities contained only the flavonoid isoquercitrin.

Results of the quantitative analysis of flavonoids are given in Table I.

The content of total flavonoids in the leaves from the two localities ranged from 0.52 to 2.00%. Significant differences ($p < 0.001$) in the content of flavonoids were found between different spots. These may be a function of various factors, such as the type of soil, microclimatic conditions, geographic position, site, age and vegetational stage of plants and leaves. Leaves collected at Božava contained a larger quantity of flavonoids compared to the leaves from Orebić (Fig. 2).

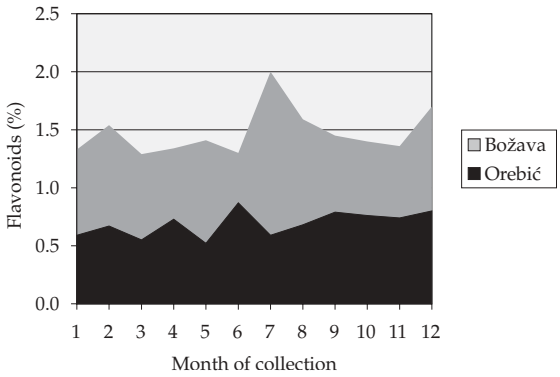

Fig. 2. Time course of the flavonoid content in the leaves at two localities.

The highest content of flavonoids in the leaves collected in Božava was found in July (2.00%), whereas the leaves collected in Orebić contained the largest quantity of flavonoids in June (0.87%). This is consistent with the oxidative pressure hypothesis that the main role of phenolics may be to protect the leaves from photodamage, not from herbivores, and they can achieve this by acting as antioxidants (7, 17–20).

The content of flavonoids in fruits collected in December was found to be 0.29% (Božava) and 0.10% (Orebić).

CONCLUSIONS

Composition and contents of flavonoids were determined in the leaves and fruits of the species *A. unedo* from two separate geographic locations in Croatia. The content of flavonoids significantly differed from one sample to another. This might be due to the various conditions of investigated plants through time and space. Further studies are needed to test the oxidative pressure hypothesis.

Acknowledgements. – This work was supported by the Ministry of Science, Education and Sports of the Republic of Croatia (Project No. 0006521).

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S A Ž E T A K

Kvalitativna i kvantitativna analiza flavonoida planike – *Arbutus unedo* L. (*Ericaceae*)

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Sastav i količina flavonoida određeni su u listovima i plodovima planike – *Arbutus unedo* L. sabranima na dva nalazišta u Hrvatskoj. Tankoslojnom kromatografijom utvrđena je prisutnost kvercitrina, izokvercitrina, hiperozida i rutina u svim uzorcima listova, dok su plodovi sadržavali samo izokvercitrin. Klorogenska kiselina je dokazana samo u nekim uzorcima listova. Količina flavonoida bila je uvjetovana biljnim organom, datumom, te zemljopisnim položajem mjesta sabiranja. Spektrofotometrijska je analiza pokazala da listovi sadrže veću količinu flavonoida (0,52–2,00%) u usporedbi s plodovima (0,10–0,29%).

Ključne riječi: *Arbutus unedo* L. (*Ericaceae*), flavonoidi, tankoslojna kromatografija, spektrofotometrija

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