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Anatomical analysis and phytochemical screening of *Frangula rupestris* (Scop.) Schur (Rhamnaceae)

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ABSTRACT: *Frangula rupestris* and *F. alnus* are the only two species of the genus *Frangula* in the flora of the Balkan Peninsula. *Frangula alnus* is well-known for anthranoid content, and its stem bark and fruits are widely used as laxatives. Data on anatomy, plant metabolites, and potential use of *F. rupestris* are scarce. In this work we analysed anatomy of the stem and leaves and performed phytochemical screening of the bark and leaves of *F. rupestris*. Specific anatomical characteristics of the stem include the presence of large mucilage cavities in the bark and pith, as well as numerous parenchyma cells containing solitary or cluster crystals of calcium oxalate. The majority of leaf epidermal cells are filled with mucilage. In the main leaf vein there is parenchyma with numerous mucilage cavities and solitary or cluster crystals of calcium oxalate. The levels of flavonoids, total phenolics, and tannins in bark and leaves of plants from two localities were determined by spectrophotometric methods, and the results were compared with those obtained for bark of *F. alnus*. Bark and leaves of *F. rupestris* contained 2.68-3.03% and 2.22-3.77% total phenolics, 1.70-2.10% and 0.57-1.54% tannins, and 0.12-0.36% and 0.57-0.99% flavonoids, respectively. The conducted HPLC and LC-MS analyses of hydromethanol extracts of bark and leaves of *F. rupestris* revealed the presence of flavonols. The dominant compound in all extracts was hyperoside and its content, determined by HPLC, ranged from 30.40 to 82.03 mg/g. Swelling indices determined for 0.5 g of plant material of *F. rupestris* bark and leaves (5.8-11.4 and 5.8-13.8) were higher than that of the bark of *F. alnus* (4.4) and greater than those of the mucilaginous drugs *Althaeae folium* and *Althaeae radix* (4.7-4.8). The high swelling indices of *F. rupestris* bark and (especially) leaves suggest their potential use as bulk-forming laxatives. In addition, differences in the content of metabolites were observed in plants from different localities.

Keywords: *Frangula rupestris*, stem, bark, leaves, anatomy, metabolites

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INTRODUCTION

The family Rhamnaceae comprises more than 500 species belonging to around 50 genera distributed throughout the world (Jovanović 1973). Several species of the genera *Rhamnus* L. (*R. cathartica* L., *R. falax* Boiss., *R. purshiana* DC.) and *Frangula* L. (*F. alnus* Mill.) are well-known for anthranoid content, and their parts, *i.e.*, stem bark and fruits, are widely used as laxatives (Kovačević 2004; Hänsel & Sticher 2010).

Frangula rupestris (Scop.) Schur (syn. *Rhamnus rupestris* Scop, *R. wulfenii* Hoppe, *Frangula wulfenii* Rchb.) and *F. alnus* are the only two species of the genus *Frangula* in the flora of the Balkan Peninsula (Tutin 1968). *Frangula rupestris* is an ascending or procumbent shrub up to 100 cm high. The young shoots are light-brown

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with inconspicuous lenticels. Leaves are 2-5 cm long, elliptical to suborbicular, with dentate or sometimes entire margins, pubescent on veins of the lower side. The flowers are small, light-green or yellowish, in umbellate inflorescences. The fruit is a round drupe, red initially, becoming purple to black during ripening. *Frangula rupestris* grows in warm, dry, limestone places, mainly in mountainous areas. It is found in the central and western regions of the Balkan Peninsula, spreading to northeastern Italy (Tutin 1968; Jovanović 1973).

Frangula alnus bark (*Frangulae cortex*) is a well-known herbal drug with longstanding use as an anthranoid laxative, and its anatomy and chemical composition have been thoroughly analysed (EMEA/HMPC/76306/2006; Hänsel & Stitcher 2010). On the other hand, available data on metabolites of *F. rupestris* are limited to several reports, mainly treating anthranoid constituents of the stem bark and leaves (Sajc *et al.* 1999; Kovačević & Grubišić 2005; Kremer *et al.* 2012). *Frangula rupestris* bark from Serbia contained 0.03% of total anthranoid aglycons, determined by HPLC after acid hydrolysis, with aloe-emodin as the most abundant, followed by chrysophanol and emodin (Sajc *et al.* 1999). Low contents of anthranoids were also found in bark samples from Croatia, i.e., 0.26-0.54% of total glucofrangulins as determined by the spectrophotometric method (Maleš *et al.* 2010). In contrast to the bark of plants from Serbia, physcion was the principle anthranoid aglycon, emodin and chrysophanol were present in lower quantities, and aloe-emodin was below the limit of quantitation in the bark sample from Croatia, analysed by HPLC after acid hydrolysis (Kremer *et al.* 2012). The amount of anthranoids in *F. rupestris* leaves from Serbia was even lower than in the corresponding bark (0.01% of total aglycons, determined by HPLC after hydrolysis). Aloe-emodin was the dominant aglycon, and chrysophanol and emodin were less abundant (Sajc *et al.* 1999).

There are sporadic data on the content of flavonoids in the bark of *F. rupestris* (Maleš *et al.* 2010; Kremer *et al.* 2012), but composition of the flavonoid fraction of neither bark nor leaves was investigated. Furthermore, data on the potential use and pharmacological effects of this species are lacking.

With these facts in mind, we set out to investigate *F. rupestris* with the aim of establishing its potential as a herbal drug source. In that connection, we examined anatomy of stem and leaves and performed phytochemical screening that included HPLC and LC-MS analyses of flavonoids; spectrophotometric determination of flavonoids, total phenolics, and tannins; and determination of the swelling indices of bark and leaves.

MATERIALS AND METHODS

Plant material. Shoots of *F. rupestris* were collected from two localities in Montenegro, Luštica and Njeguši, in August of 2015, whereas *F. alnus* shoots were collected in Serbia on Mt. Goč in June of 2005. Voucher specimens were deposited in the herbarium of the Institute of Botany and Botanical Garden "Jevremovac", University of Belgrade – Faculty of Biology (BEOU); and in that of the Department of Botany, University of Belgrade – Faculty of Pharmacy (HFF) (Table 1).

Table 1. Plant material used in the study.

Species	Locality	Plant part	Voucher number
Frangula rupestris	Luštica	Stem	42962 BEOU
		Leaves	
	Njeguši	Stem	42970 BEOU
		Leaves	
Frangula alnus	Goč	Bark	1465 HFF

Analysis of stem, petiole, and leaf anatomy. Anatomical analyses were done on the sample collected at the Luštica locality. Fresh plant material was fixed in 50% ethanol. Cross-sections of the stems and leaves (up to 20 μm thick) were made on a Reichert sliding microtome. The sections were cleared in Parazone and thoroughly washed before staining in safranin solution (1% w/v in 50% ethanol) and alcian blue solution (1% w/v in water). After that, the cross-sections were passed through a series of of ethanol solutions of increasing concentration (50, 70, 96, and 100%). All slides were mounted in Canada balsam after dehydration.

Epidermal peels for examination of surface structures were prepared using Jeffrey's solution (10% nitric acid and 10% chromic acid, 1:1). All slides were mounted in glycerol after dehydration. All histological slides were examined and photographed using an Olympus BX41 light microscope.

Determination of flavonoid content. A spectrophotometric method based on the Wilson-Taubőck reaction was used to determine the content of total flavonoids in the plant material. In the presence of boric and oxalic acids, flavonoids form fluorescent yellow complexes. Absorbance was subsequently measured at 410 nm (Petrović *et al.* 2013). The procedure was conducted as described in the Ph. Eur. monograph on *Crataegi folium cum flore* (Ph. Eur. 2013). The results were calculated using the specific absorption coefficient of the hyperoside complex and expressed as % *m/m*.

Determination of tannin and total phenolic content. The content of total phenolics in plant material was determined by the spectrophotometric FC (Folin-Cio-

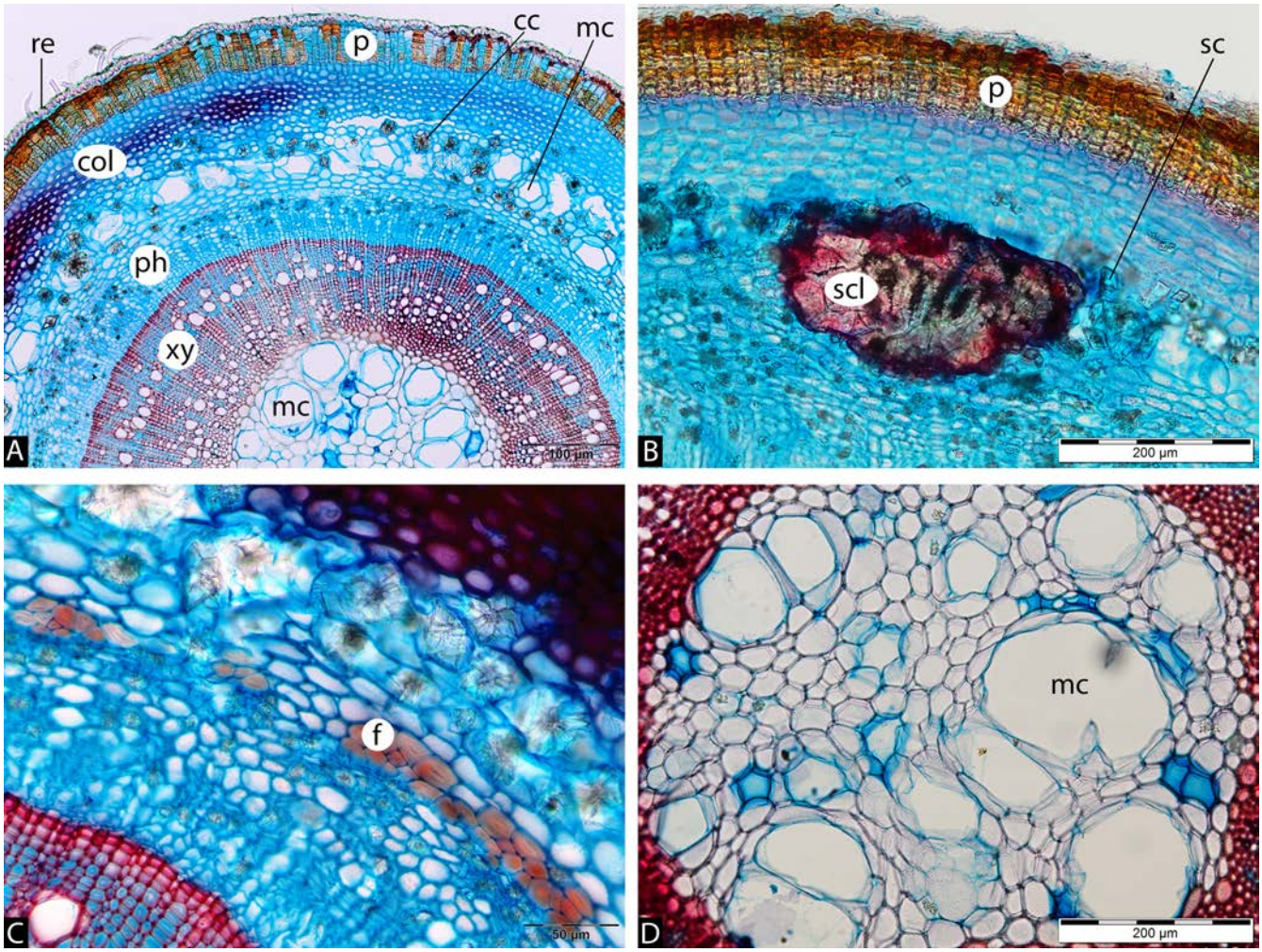

Fig. 1. Cross section of the secondary stem: A) whole stem; B & C) bark; D) pith (**re** – residual epidermis; **p** – periderm; **col** – collenchyma; **mc** – mucilage cavities; **cc** – cluster crystals; **ph** – phloem; **xy** – xylem; **sc** – solitary crystals**; scl** – sclereids; **f** – fibres).

calteu) method. Phenolic compounds react with FC reagent (phosphomolybdic/phosphotungstic acid complexes) to produce the blue colour of reduced molybdenum and tungsten with an absorption maximum at 760 nm. The amount of tannins was determined after precipitation with hide-powder. Phenolic compounds unadsorbed on hide powder (nontannin phenolics) were determined with FC reagent and tannin content was obtained from the difference in content of total and nontannin phenolics (Petrović *et al.* 2013). The procedure was conducted according to Ph. Eur. (*2.8.14*) (Ph. Eur. 2013), the results being expressed in pyrogallol equivalents (% *m/m*).

Preparation of extracts. For the preparation of dry hydromethanol extracts (ME), samples of bark and leaves of *F. rupestris* were air-dried and powdered. Subsequently, plant material (2 g) was sonicated for 15 min with 70% methanol (*v/v* 30 mL) and macerated for 24 h. After filtration and evaporation of the solvent, the ME yields were 15.0% (Luštica) and 12.6% (Njeguši) for bark samples, and 22.5% (Luštica) and 17.5% (Njeguši) for leaf samples.

Liquid chromatography (LC). High-pressure liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC-MS) were conducted for analysis of flavonoid constituents in MEs.

Qualitative and quantitative analyses by HPLC were performed on an Agilent 1100 liquid chromatograph equipped with a photodiode array detector (DAD) using a Zorbax Eclipse XDB-C18 analytical column (4.6 × 250 mm; 5 µm particle size). Batches of MEs (5 mg/mL in 70% methanol) were gradiently eluted in a a two-phase system (phase A - 0.03% phosphoric acid; and phase B - 10% A in acetonitrile) at a flow rate of 0.8 mL/min and temperature of 25°C. The gradient profile was as follows: 10-25% B, 5 min; 25% B, 10 min; 25-30% B, 5 min; 30-50% B, 5 min; 50-70% B, 5 min; and return to initial conditions for 5 min. Chromatograms were recorded at 210, 320, 350, and 370 nm. Identification of constituents

Table 2. Content of flavonoids, tannins, and total phenolics in bark and leaves of *F. rupestris* and bark of *F. alnus*.

	Content (%)		
Sample	**Flavonoids**[a]	**Tannins**[b]	**Total phenolics**[b]
F. rupestris bark – Luštica	0.36	2.10	3.03
F. rupestris bark – Njeguši	0.12	1.70	2.68
F. rupestris leaves – Luštica	0.99	1.54	3.77
F. rupestris leaves – Njeguši	0.57	0.57	2.22
F. alnus bark – Goč	0.15	2.92	4.40

Results are presented as the mean of two independent assays. a – expressed as hyperoside; b – expressed as pyrogallol.

Table 3. Results of HPLC and LC-MS analyses of *F. rupestris* bark and leaf extracts.

				Content (mg/g)[b]			
		UV data	**MS data**	**Bark**		**Leaves**	
R_t[a] (min)		**λ_{max} (nm)**	**(*m/z*)**	**Luštica**	**Njeguši**	**Luštica**	**Njeguši**
7.04	Quercetin glycoside	354, 268sh, 256	917 $[M-H]^-$	n.d.	n.d.	3.89	4.14
7.72	Quercetin glycoside	360, 268sh, 256	959 $[M-H]^-$	n.d.	n.d.	5.25	**19.69**
8.22	Kaempferol glycoside	350, 266	943 $[M-H]^-$	n.d.	n.d.	3.74	5.12
8.56	Quercetin glycoside	358, 300, 270sh, 250	973 $[M-H]^-$	n.d.	n.d.	n.d.	9.56
9.91	Quercetin glycoside	356, 300, 268sh, 256	755 $[M-H]^-$, 301	**9.76**	**8.33**	**11.44**	6.85
10.31	Rutin (Quercetin 3-*O*-rutinoside)	354, 298, 268sh, 256	609 $[M-H]^-$, 301	**15.47**	**12.05**	8.83	4.99
11.32	Hyperoside (Quercetin 3-*O*-galactoside)	358, 298, 268sh, 256	463 $[M-H]^-$	**61.97**	**30.40**	**82.03**	**49.32**
13.09	Kaempferol hexoside	350, 266	447 $[M-H]^-$, 285	tr.	tr.	**11.61**	**7.63**
13.76	Quercetin hexuronide	360, 268sh, 254	477 $[M-H]^-$, 301	1.71	5.17	n.d.	n.d.
14.56	Quercitrin (Quercetin 3-*O*-rhamnoside)	356, 298, 268sh, 254	447 $[M-H]^-$, 301	n.d.	n.d.	1.22	6.63
25.60	Quercetin dimethyl ether hexoside	358, 268sh, 254	491 $[M-H]^-$, 329	2.01	2.24	n.d.	n.d.
26.86	Quercetin	372, 300, 272sh, 256	301 $[M-H]^-$	1.96	4.28	**20.45**	**12.60**
27.51	Quercetin 3-methyl ether	354, 302, 288, 268sh, 256	315 $[M-H]^-$, 300	2.11	1.83	n.d.	n.d.
28.20	Quercetin dimethyl ether	358, 298, 270sh, 256	329 $[M-H]^-$	1.37	0.86	7.74	2.07
29.28	Kaempferol	366, 266, 250sh, 294sh, 320sh	285 $[M-H]^-$	n.d.	0.43	7.09	3.27
29.61	Isorhamnetin (Quercetin 3'-methyl ether)	372, 328sh, 302sh, 254	315 $[M-H]^-$	n.d.	n.d.	n.d.	4.10
30.08	Quercetin dimethyl ether	356, 294sh, 268sh, 254	329 $[M-H]^-$	0.58	1.04	0.41	n.d.
	Total			**96.9**	**66.6**	**163.7**	**136.0**

a – retention times obtained by HPLC; b – determined by HPLC method and calculated as hyperoside; tr. – trace; n.d. – not detected.

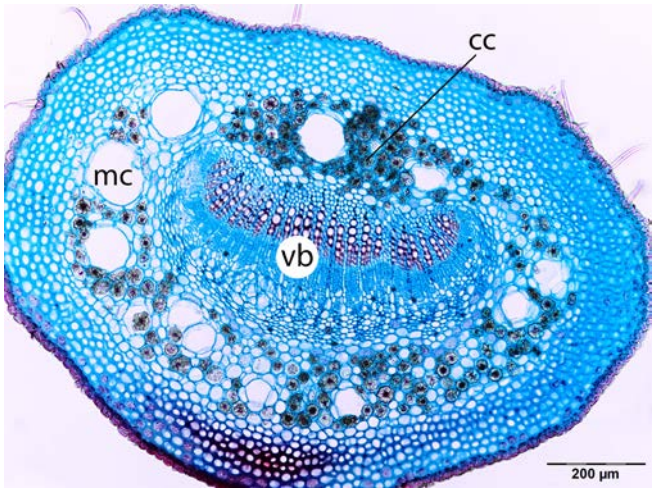

Fig. 2. Cross section of the petiole (**mc** – mucilage cavites; **cc** – cluster crystals; **vb** – vascular bundle).

was performed by comparing their retention times and UV spectra to those of available standards. Ultraviolet spectra were also compared with published data (Mabry *et al.* 1970; Pavlović 2008). The content of flavonoid constituents was determined at 350 nm by the external calibration method using serial dilutions (0.02-0.36 mg/mL) of hyperoside (Carl Roth, Germany) as the standard compound ($Y = 20036 \cdot X + 146$, $R^2 = 0.997$).

Qualitative analysis of the extracts was also performed by LC-MS on an Agilent 1260 liquid chromatograph equipped with an auto sampler and a PDA detector, and coupled with an Agilent MSD 6100 single-quad mass detector (United States). Separation was done on a Zorbax SB-aq column (150 × 2.1 mm; 3.5 µm particle size) maintained at 27°C. Solutions of ME (3 µL, 5 mg/mL in 70% methanol) were injected and the gradient elution was performed at a flow rate of 0.3 mL/min using mobile phase A (0.1% formic acid) and mobile phase B (acetonitrile) as follows: 10-60% B 0-25 min, 60-95% B 5 min, and return to initial conditions for the next 2 min. Chromatograms were recorded at 210, 280, 320, 350, and 370 nm. The analysed ESI-MS spectra were obtained in the negative mode in a range of 150-850 *m/z* or 150-1150 *m/z*. The ion chamber was maintained at 350°C with nebulization under conditions of a nitrogen flow rate of 10 L/min, pressure of 30 psi, and capillary voltage of 3500 V. Signals were recorded by applying fragmentor voltage of 100, 200, or 250 V.

Determination of swelling index. The swelling index is the volume in millilitres occupied by a defined mass of plant material, including any adhering mucilage, after it has swollen in water for 4 h (Ph. Eur. 2013). Determination of swelling indices for 0.5 g of powdered plant material was performed in accordance with the procedure given in Ph. Eur. (*2.8.4*) (Ph. Eur. 2013). For purposes of comparison, the swelling indices of *F. alnus* bark and commercial samples of the mucilaginous herbal drugs *Althaeae radix* and *Althaeae folium* (Institute for Medicinal Plant Research "Dr Josif Pančić", Belgrade) were determined for 0.5 g also.

RESULTS AND DISCUSSION

Anatomy of stem, petiole, and leaves

The perennial stem of *F. rupestris* consists of bark, cambium, and wood. Somewhere on the surface of the stem there are residuals of the epidermis (Fig. 1A), while on older stems there is only periderm (Fig. 1B). The bark is composed of periderm, multilayer collenchyma, and parenchyma with numerous mucilage cavities. Many cells of parenchyma contain solitary or cluster crystals of calcium oxalate (Fig. 1A, B). In the bark parenchyma of older stems, there are smaller or larger groups of sclereids (Fig. 1B). Above the phloem, there are pericycle residues consisting of groups of fibees and parenchyma cells (Fig. 1C). Parenchyma cells of phloem and cortical rays contain solitary or cluster crystals of calcium oxalate. Secondary wood consists of xylem and pith. Xylem consists of vessels, *i.e.*, tracheas and tracheids of various sizes, and medullary rays 1-3 cells wide (Fig. 1A). Large mucilage cavities are present in the pith (Fig. 1D). Some parenchyma cells of pith contain cluster crystals of calcium oxalate.

Microscopic examination indicated that the high proportion of sclereids, fewer fibres (which are located only above the phloem zone), and the presence of large calcium oxalate clusters are useful characters for distinguishing the bark of *F. rupestris* from that of *F. alnus*, whose characteristics were described previously (Metcalfe & Chalk 1950).

The petiole consists of epidermis, collenchyma, parenchyma, and the vascular bundle. Mucilage cavities are widespread throughout the parenchyma. Many parenchyma cells contain cluster crystals of calcium oxalate (Fig. 2).

Leaves have a single-layered epidermis. Anticlinal walls of the adaxial and abaxial epidermal cells are slightly undulate (Fig. 3A). Stomata are of the anomocytic type and present only on the abaxial side (Fig. 3Ab). The leaves are dorsoventral. Cells of the adaxial epidermis are larger than those of the abaxial epidermis. The majority of epidermal cells are filled with mucilage (Fig. 3B, C). The mesophyll consists of multilayer palisade and spongy tissues. In the main vein below the phloem, there is parenchyma with numerous mucilage cavities and solitary or cluster crystals of calcium oxalate (Fig. 3C).

Phytochemical screening of *F. rupestris* stem bark and leaves

Content of flavonoids, tannins, and total phenolics. The results of quantitative analysis of the investigated parts of *F. rupestris* are presented in Table 2. For purposes of

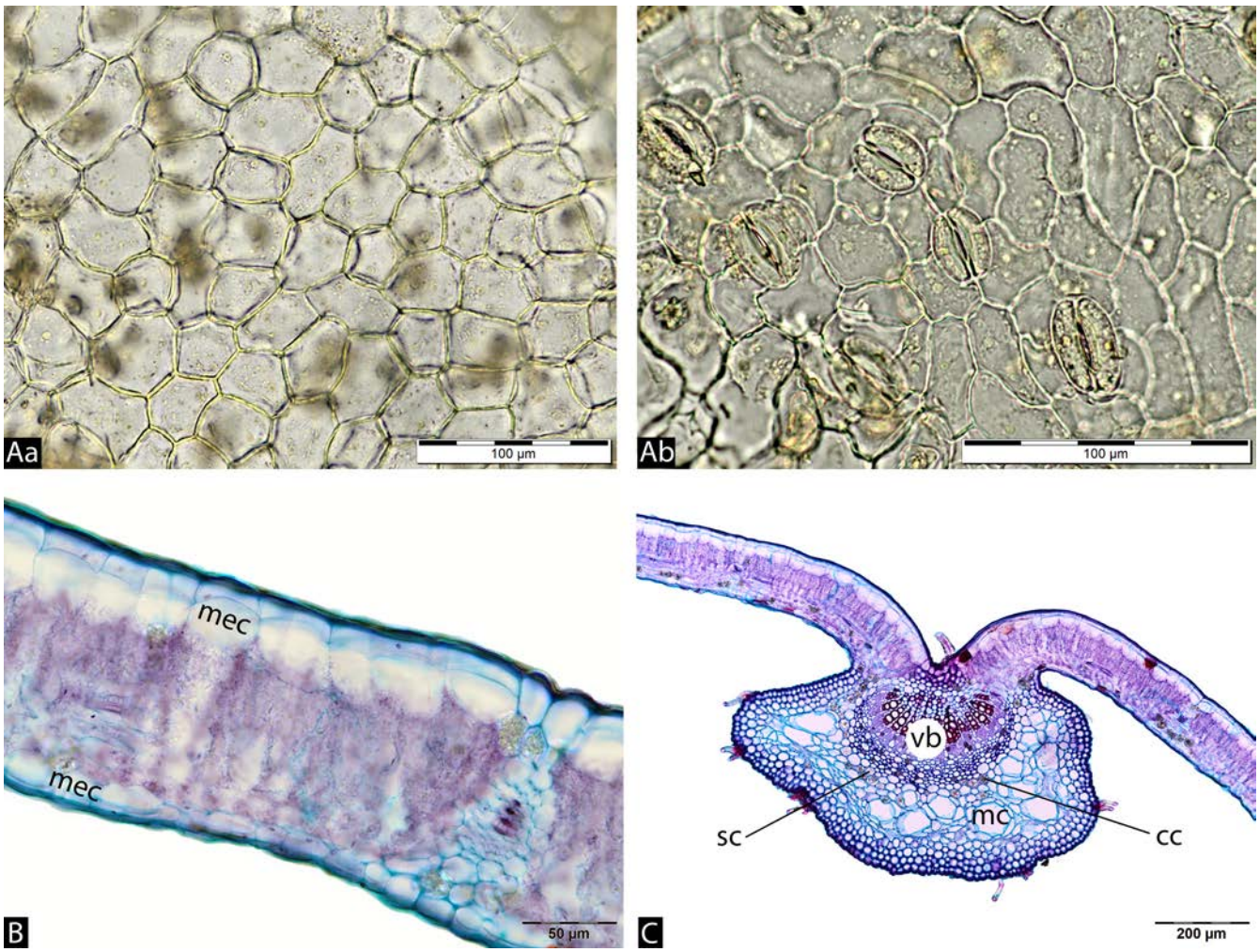

Fig. 3. Leaves: A) paradermal section (**a** – adaxial epidermis; **b** – abaxial epidermis); B & C) cross section (**mec** – mucilage epidermal cells; **mc** – mucilage cavities; **cc** – cluster crystals; **sc** – solitary crystals**; vb** – vascular bundle).

comparison, the bark of *F. alnus* was also investigated in the same manner.

The content of flavonoids was higher in leaf samples of *F. rupestris* (0.57-0.99%), than in bark samples of both *F. rupestris* (0.12-0.36%) and *F. alnus* (0.15%). In previously analysed barks of these two species from Croatia, the content of flavonoids (determined by a method based on the reaction of flavonoids with $AlCl_3$) amounted to 0.06-0.08% (Maleš *et al*. 2010).

Unlike flavonoids, tannins had greater content in bark samples of both species (1.70-2.92%) than in leaf samples of *F. rupestris* (0.57-1.54%).

The highest level of total phenolics was determined in *F. alnus* bark. *Frangula rupestris* leaves and bark from Luštica had greater amounts of total phenolics than bark and leaf samples from Njeguši. In addition, the bark of *F. rupestris* contained somewhat smaller amounts of total phenolics (2.68-3.03%) and tannins (1.70-2.10%) than the bark of *F. alnus* (4.40% and 2.92%, respectively). The values of tannin content in the barks investigated in the present study were in accordance with previously analysed barks of these two species (1.44-2.69%). On the other hand, the level of total phenolics was lower in the barks investigated here than in samples from Croatia (4.09-5.24% in *F. rupestris* and 5.57-8.30% in *F. alnus*) (Maleš *et al*. 2010).

LC analyses of flavonoids. Our HPLC and LC-MS analyses revealed the presence of flavonols in bark and leaf extracts of *F. rupestris* (Table 3). The UV, MS, and Rt data of detected compounds corresponded to those of the flavonol aglycones quercetin, quercetin methyl ethers, kaempferol, and their glycosides. The presence of quercetin, kaempferol, and isorhamnetin (quercetin 3'-methyl ether), as well as hyperoside (quercetin 3-*O*-galactoside), rutin (quercetin 3-*O*-rutinoside), and quercitrin (quercetin 3-*O*-rhamnoside), was confirmed by HPLC using reference compounds.

The results of quantitative HPLC analysis are presented in Table 3. The dominant compound in all extracts was hyperoside, ranging in content from 30.40 mg/g in

Table 4. Swelling indices (determined for 0.5 g of plant material)

Sample	Swelling index
F. rupestris bark – Luštica	5.0
F. rupestris bark – Njeguši	11.4
F. rupestris leaves – Luštica	5.8
F. rupestris leaves – Njeguši	13.8
F. alnus bark – Goč	4.4
A. officinalis root (*Althaeae radix*)	4.8
A. officinalis leaves (*Althaeae folium*)	4.7

Results are presented as the mean of two independent determinations.

the bark extract originating from Njeguši to 82.03 mg/g in the leaf extract from Luštica. In bark extracts, hyperoside was followed by rutin (12.05-15.47 mg/g calculated as hyperoside). Leaf extracts, in addition to hyperoside, were rich with the aglycone quercetin (12.60-20.45 mg/g calculated as hyperoside) and highly polar quercetin glycosides. Leaf extracts were also characterised by significant amounts of a kaempferol hexoside.

Flavonoid profiles of *F. rupestris* bark and leaves were not investigated anywhere previously, and in the current study we confirmed the presence of flavonol aglycones and their glycosides. This class of compounds was previously found in the aerial parts of several other species of the family Rhamnaceae. The quercetin methyl ether isorhamnetin, as well as rhamnosin (3',7-di-*O*-methyl quercetin), 3-*O*-methyl quercetin, and rhamnocitrin (7-*O*-methyl kaempferol), were isolated from the aerial parts of *Rhamnus lycoides* (Payá *et al.* 1986). The glycosides 7-*O*-methylkaempferol-3-*O*-rhamnoside and kaempferol-3-*O*-rhamnoside were found in leaves and twigs of *R. virgatus* (Prasad *et al.* 2000). Leaves of *R. alaternus* contained rutin, kaempferol-3-*O*-isorhamninoside, rhamnocitrin-3-*O*-isorhamninoside, and rhamnetin-3-*O*-isorhamninoside, along with the aglycones kaempferol and quercetin and the flavone apigenin (Ben Ammar *et al.* 2009; Moussi *et al.* 2015).

Swelling index. In agreement with previous findings indicating the presence of mucilage structures in the stem and leaves of the related *F. alnus* (Nikitin & Pankova 1982), the anatomical analysis conducted in the current study revealed the presence of numerous mucilage-containing structures in the stem and leaves of *F. rupestris*. This led to the presumption that the investigated plant material should exhibit significant swelling ability.

Leaf samples of *F. rupestris* swelled in a similar manner or even better than did leaf samples of *Althaea officinalis*, a well-known demulcent herbal drug that was used for comparison. The same observation is valid for bark samples of *F. rupestris* in comparison with the sample of *F. alnus* bark and a commercial root sample of the mucilaginous drug *A. officinalis* (Table 4).

The leaf sample of *F. rupestris* swelled better than the bark sample from the same locality. Furthermore, the bark and leaf samples from Njeguši had exceptionally high swelling indices in comparison with the bark and leaf samples from Luštica (Table 4).

Mucilages can have a variety of functions, such as germination and dispersal, maintaining water status, and aiding freezing tolerance, or they may serve as storage material. In wood and bark of plants under high saline or drought conditions, they have an important role in maintaining sufficient water tension. Leaf mucilages can offset water stress by absorbing water from the atmosphere (Hänsel & Sticher 2010; Patten *et al.* 2010). In that regard, it can be assumed that the observed difference between swelling indices of bark samples and especially leaf samples of *F. rupestris* from two localities may be related to different habitat conditions and adaptation of the plants to their environment. However, broader study is necessary for confirmation of this assumption.

If we compare the phytochemical profiles of *F. rupestris* samples originating from different localities, we note that the bark and leaves of samples from Luštica were to some extent richer with phenolic compounds (total phenolics, tannins, and flavonoids) than the bark and leaf samples from Njeguši. On the other hand, *F. rupestris* bark and leaves from Njeguši exhibited exceptional swelling ability.

CONCLUSION

In the current study, we determined high swelling indices of bark and leaves of *F. rupestris*, accompanied by low to moderate amounts of flavonoids, tannins, and total phenolics. It was previously shown that low levels of anthranoids restrain the use of bark of *F. rupestris* as a stimulant laxative. However, the high swelling index of *F. rupestris* bark and (especially) leaves indicates the expediency of conducting further investigations to establish the possibility of using them as demulcent herbal drugs, preferably as bulk-forming laxative agents. The fact that bark and leaves of *F. rupestris* are not rich in tannins (*i.e.*, compounds with astringent and antidiarrheal activity) may be favourable in that regard.

Furthermore, the observed differences in the content of metabolites in plants from different localities should be taken into consideration when collecting plant material from wild populations.

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REZIME

Anatomska analiza i fitohemijski skrining vrste *Frangula rupestris* (Scop.) Schur (Rhamnaceae)

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Frangula rupestris i *F. alnus* (Rhamnaceae) su jedini predstavnici roda *Frangula* u flori Balkanskog poluostrva. Dok je vrsta *F. alnus* dobro poznata po sadržaju antranoida, zbog čega se kora i plod koriste kao laksansi, podaci o anatomiji, metabolitima i mogućnostima primene *F. rupestris* su nepotpuni. U radu je ispitana anatomija stabla i lista i sproveden fitohemijski skrining kore i lista *F. rupestris*. Specifične anatomske karakteristike stabla su krupne sekretorne šupljine sa sluzi u kori i srži, kao i mnogobrojne parenhimske ćelije sa kristalima kalcijum oksalata u obliku druza ili pojedinačnih kristala. Većina epidermalnih ćelija lista sadrže sluzi. U listu, u zoni centralnog nerva, su krupne sekretorne šupljine sa sluzi, kao i parenhimske ćelije sa kristalima kalcijum oksalata. Sadržaj flavonoida, ukupnih polifenola i tanina, u uzorcima kore i lista sa dva lokaliteta, određen je spektrofotometrijskim postupcima i rezultati su upoređeni sa rezultatima dobijenim za koru *F. alnus*. Kora i list *F. rupestris* sadržali su 2,68-3,03% i 2,22-3,77% ukupnih polifenola, 1,70-2,10% i 0,57-1,54% tanina, i 0,12-0,36% i 0,57-0,99% flavonoida, redom. HPLC i LC-MS analizom vodeno-metanolnih ekstrakata kore i lista *F. rupestris*, pokazano je prisustvo flavonola. Dominantno jedinjenje u svim ekstraktima bio je hiperozid čiji je sadržaj, određen HPLC metodom, iznosio 30,40-82,03 mg/g. Broj bubrenja, određen za 0,5 g biljnog materijala, za koru (5,8-11,4) i list (5,8-13,8) *F. rupestris*, bio je veći broja bubrenja kore *F. alnus* (4,4), kao i od broja bubrenja sluznih droga *Althaeae radix* i *Althaeaae folium* (4,7-4,8). Visoke vrednosti broja bubrenja kore i, naročito, lista *F. rupestris* ukazuju na mogućnost njihove primene kao zapreminskih laksativa. Pored toga, uočene su i razlike u sadržaju metabolita biljaka sa različitih lokaliteta.

Ključne reči: *Frangula rupestris*, stablo, kora, list, anatomija, metaboliti