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Ana TOPALOVIĆ[1], Maja MIKULIČ-PETKOVŠEK[2], Natalija PEROVIĆ[1], Snežana TRIFUNOVIĆ[3] and Mirko KNEŽEVIĆ[1]

PHENOLIC COMPOSITION OF THE LEAF OF GRAPEVINE CV. 'CARDINAL'

SUMMARY

HPLC/DAD/ESI/MS analyses were performed to determine the phenolic composition of leaf blade and petiole of the grapevine cv. 'Cardinal'. The following compounds were identified: the hydroxycinnamic acid derivatives – caftaric acid and coutaric acid, a derivative of sinapic acid and glucose and a derivative of *p*-coumaric acid and hexose; flavonols – quercetin-3-rutinoside, quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-glucuronide, kaempferol-3-rutinoside, kaempferol-3-galactoside, kaempferol-3-glucoside and kaempferol-3-arabinoside; and flavan-3-ols – catechin, epicatechin, and procyanidin dimers, as well as procyanidin B1 and B2. The leaf blade was more abundant in phenolic compounds than the petiole. The leaf blade and the petiole had the highest concentration of quercetin-3-glucuronide.

Keywords: blade, petiole, phenolic compounds

INTRODUCTION

Fruits and vegetables are important functional foods because they represent a source of biologically active substances, which have an impact on the metabolism and certain body functions and contribute to health promotion. In addition to the well-known natural antioxidants, such as vitamins C and E, carotenoids and the minerals Se and Zn, phenolic compounds have a special role in health protection and disease prevention. Phenolic compounds can be classified into two groups: nonflavonoids (hydroxybenzoic and hydroxycinnamic acids) and flavonoids (anthocyanins, flavonols and flavan-3-ols). Flavonols and flavan-3-ols are known for their *in vitro* antioxidant activity, which in certain compounds is even higher than the activity of vitamins C and E. There have been many studies on the phenolic composition of the grape skin and the seeds of wine varieties, but there is little published information on table grape varieties (Cantos *et al.*, 2002; Rusjan *et al.*, 2008; Topalović *et al.*, 2011). In addition to the studies of the fruit itself, the phenolic composition of the other parts of the grapevine

[1]Ana TOPALOVIĆ, M.Sc. (corresponding author: anato@ac.me), Natalija PEROVIĆ, Ph.D., Mirko KNEŽEVIĆ, Ph.D., Biotechnical Faculty, University of Montenegro, Mihaila Lalića 1, 81000 Podgorica, Montenegro;

[2] Maja MIKULIČ-PETKOVŠEK, Ph.D., Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

[3] Snežana TRIFUNOVIĆ, Ph.D., Faculty of Chemistry, University of Belgrade, Studentski trg 16, 11158 Beograd, Serbia

have also been studied. Such studies are very important due to the nutritional value of grapes and their use in medicine (Hmamouchi *et al.*, 1996, Doshi *et al.*, 2006). In certain European countries, the leaves of the grapevine (*Vitis vinifera* L.) are traditionally used as a food, fresh or pickled, and also for treatment of a various ailment, such as hypertension, diarrhoea, varicose veins and inflammatory diseases (Dani *et al.*, 2010). Studies of the antioxidative activities of the grapevine have shown that the leaf petioles and blades have two to three times higher antioxidative ability compared with the grape (Doshi *et al.*, 2006). As a rich source of phenolic compounds, and due to high antioxidative capacity, the grapevine leaf is utilized in the pharmaceutical, cosmetic and food industries.

The aim of this paper was to investigate the phenolic composition of the grapevine leaves of the 'Cardinal' cultivar, as well as the ratios of phenolic compounds in the blades and petioles.

MATERIAL AND METHODS

During the grape ripening phase ('Cardinal' on Paulsen rootstock, plantation from 1996), grapevine leaves were gathered from the middle part of the shoots facing the cluster. Upon sampling (20 samples in total), the leaf blade was separated from the petiole. The samples were maintained in a freezer at -20 °C until analysis.

Phenolic compounds extraction: The frozen samples were crushed with a mortar and pestle using liquid nitrogen. The samples were prepared for HPLC analysis of the phenolic compounds by treating 0.5 g of the leaf blade with 10 ml and 0.5 g of the petiole with 3 ml of methanol containing 1% 2,6-di-*tert*-butyl-4-methylphenol (BHT) and 3% formic acid. The extraction was performed in an ultrasound bath for one hour, adding ice occasionally. Following the extraction, the samples were centrifuged for seven minutes at 10,000 rpm at a temperature of 0 °C. The supernatants were filtered through polyamide filters of 0.45 μm (Macherey Nagel, Duren, Germany), transferred into the vials and maintained at -20 °C until the HPLC analysis.

Determination of the concentration of the phenolic compounds: The HPLC analysis of the phenolic compounds was carried out with the Surveyor HPLC system (Thermo Scientific, San Jose, CA) equipped with a diode array detector (DAD). A Column Gemini C18 (150 x 4.6 mm; 3 μm; Phenomenex, Torrance, USA) was used with the precolumn Phenomenex at a temperature of 25 °C. The mobile phase A was 1% formic acid, while the mobile phase B was 100% acetonitrile. The flow rate was 1 ml/min, using the gradient programme described by Marks *et al.* (2007). Flavan-3-ols and hydroxycinnamic acids were analysed at 280 nm and flavonols at 350 nm. The injection volume of extract was 20 μl. The phenolic compounds were identified by comparing the retention times and their UV-Vis spectra from 200 to 600 nm, as well as by adding the appropriate standard solution. Representative chromatograms are presented in Figure 1.

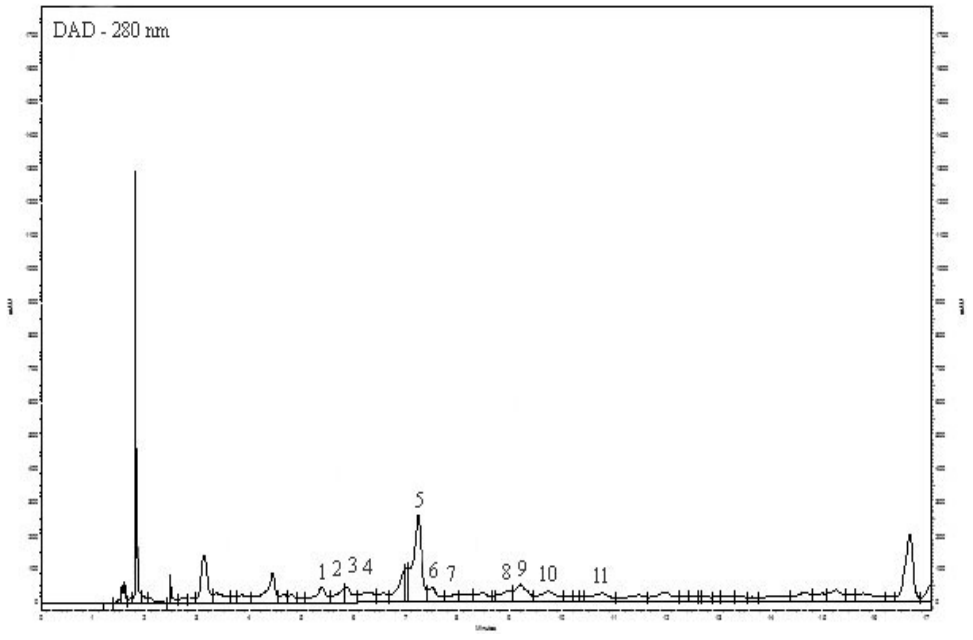
Mass spectra were obtained using the LCQ Deca XP MAX (Thermo Scientific, Waltham, Massachusetts, USA), with an electrospray interface (ESI) operating in negative ion mode. The analyses were carried out using the full-scan data dependent MS^2 scanning from 115 to 2000 *m/z* (Table 1). The capillary temperature was 250 °C, the sheath gas and auxiliary gas were 20 and 7 units, respectively, and the source voltage was 0.1 kV for negative ionization.

Table 1. Phenolic compounds identified in a grapevine leaf using HPLC-MS and MS^2

Peak no.	λ (nm)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Compound	Expressed as
1	280	577	425, 407, 289	Procyanidin dimer 1	Procyanidin B1
2		577	425, 407, 289	Procyanidin dimer 2	Procyanidin B1
3		577	425, 407, 289	Procyanidin dimer 3	Procyanidin B1
4		577	425, 407, 289	Procyanidin B1	Procyanidin B1
5		311	179,149	Caftaric acid	*p*-Coumaric acid
6		325	163, 145	*p*-Coumaroyl-hexose	*p*-Coumaric acid
7		289	245	Catechin	Catechin
8		577	425, 407, 289	Procyanidin B2	Procyanidin B2
9		385	223, 205, 153, 161	Derivative of sinapic acid and glucose	Sinapic acid
10		295	163	Coutaric acid	*p*-Coumaric acid
11		289	245	Epicatechin	Epicatechin
12	350	609	301	Quercetin-3-*O*-rutinoside	Quercetin-3-*O*-rutinoside
13		463	301	Quercetin-3-*O*-galactoside	Quercetin-3-*O*-galactoside
14		463	301	Quercetin-3-*O*-glucoside	Quercetin-3-*O*-glucoside
15		477	301, 179, 151	Quercetin-3-*O*-glucuronide	Quercetin-3-*O*-galactoside
16		593	285	Kaempferol-3-*O*-rutinoside	Kaempferol
17		447	285	Kaempferol-3-*O*-galactoside	Kaempferol
18		447	285	Kaempferol-3-*O*-glucoside	Kaempferol
19		417	285	Kaempferol-3-*O*-arabinoside	Kaempferol

The concentrations of the phenolic compounds were calculated using the peak areas of the sample and the relevant standards. In the absence of authentic substances, the quantification of certain phenolic compounds was performed using the compounds of a similar structure.

a)



b)

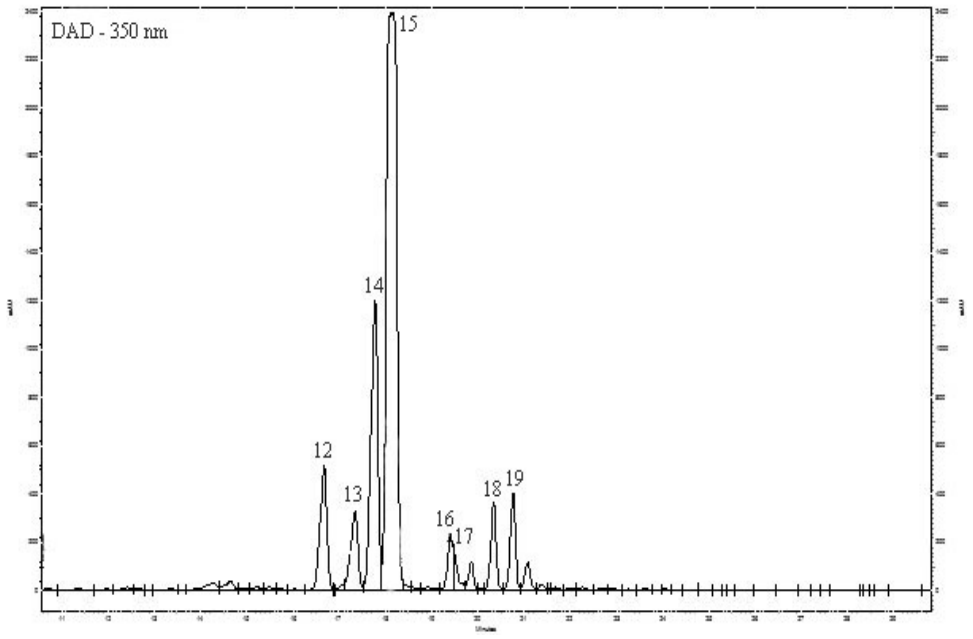


Figure 1. Chromatograms for the leaf blade: a) flavan-3-ols, b) flavonols

RESULTS AND DISCUSSION

The grapevine leaf and the petiole, in particular, are used to determine the status of nutrients in the grapevine, most frequently during the full flowering stage. Taking into account the significance of the leaf as a photosynthetic organ, and as a follow-up to previous research work monitoring certain metabolites in grapes (sugars, organic acids and phenolic compounds) during ripening, (Topalović and Mikulič-Petkovšek, 2010; Topalović *et al.*, 2011), the phenolic composition of the grapevine leaf was analysed.

HPLC-DAD/ESI-MS analyses of the grapevine leaf resulted in identification of hydroxycinnamic acid derivatives (bound forms), flavan-3-ols and flavonols (Table 1). The analysed leaf parts (the leaf blade and the petiole) contained the highest concentration of quercetin-3-glucuronide, with the relative abundance amounting even to approximately 36% in the leaf blade and 18% in the petiole (Figure 2). Generally, among the flavonols, quercetin-3-glucoside and rutin had relatively high concentrations. Similar results have been obtained in other studies. Monagas *et al.* (2006) underlined that quercetin-3-glucuronide was the most commonly represented flavonol in commercial dietary ingredients obtained from the grapevine leaf. Doshi *et al.* (2006) established that the leaf of the grapevine cultivar 'Sharad Seedless' contains more flavonol than other parts (shoots, berry stem, grapes and petiole). Hmamouchi *et al.* (1996) identified ten flavonoids in some Moroccan cultivars of grapevine (*Vitis vinifera* L.), with the most common being quercetin-3-glucuronide at a concentration of 2500 to 8500 mg/kg, quercetin-3-glucoside at a concentration of 2200 to 6200 mg/kg and quercetin-3-rutinoside at a concentration of 1300 to 4200 mg/kg. The concentrations of the phenolic compounds in the Moroccan cultivars refer to the dry matter, explaining why these are significantly higher compared with the cultivar 'Cardinal' (Table 2).

Besides the flavonols mentioned above, it has been established that the leaf blade is also rich in procyanidin dimers. The situation is similar with the petiole, except that the petiole is also relatively rich in catechin and epicatechin. The concentrations of other phenolic compounds are low.

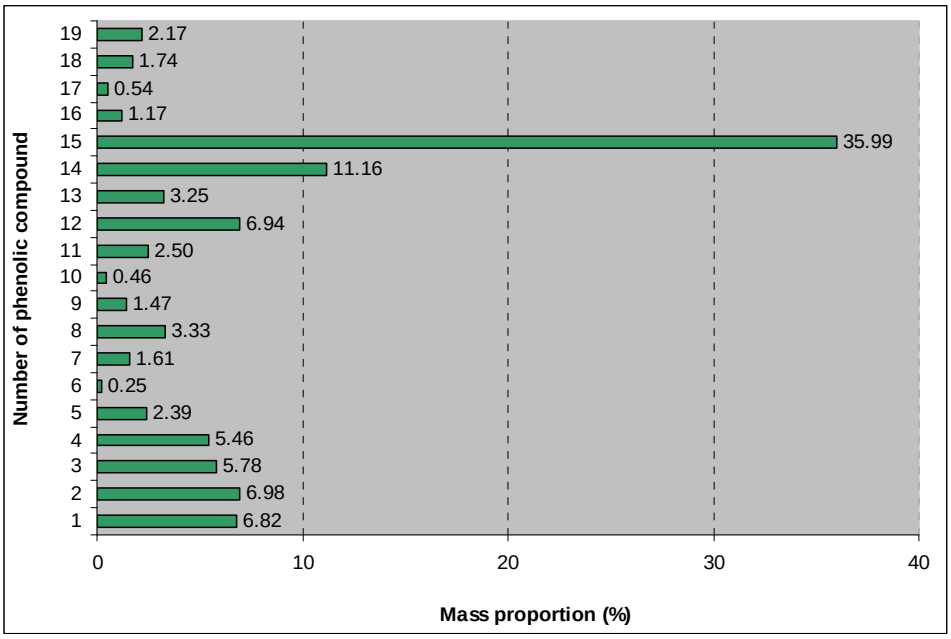
The grapevine leaf blade contained much more phenolic compounds (about five times more, by the sum of concentrations) compared with the petiole. Only the content of catechin was higher in the petiole than in the leaf blade. Among the phenolic compounds analysed, kaempferol glycosides (the flavonols with relatively small mass proportion in the grapevine leaf) had the highest value (12–21) in concentration ratios (Figure 2). The concentration of quercetin glycosides (the flavonols with a relatively high mass proportion in the grapevine leaf) was 7–12 times higher in the leaf blade than in the petiole. Flavan-3-ols had 2–4 times higher, whereas the concentration of derivatives of hydroxycynammic acid was 2–6 times higher in the leaf blade than in the petiole. The results indicate that the antioxidative activity of the grapevine leaf blade would be higher than the petiole and that the leaf blade would, thus, be more important in the production of dietary supplements. Previous work has already established that

procyanidins have strong antioxidative activity *in vitro* (Yamaguchi *et al.*, 1999) and *in vivo* (Koga *et al.*, 1999). Various properties are ascribed to plant flavonoids, such as anti-inflammatory, antihepatotoxic, antihypertensive, antirheumatic, antiallergic and antitumor activities etc. The results of an eight-year study showed that the presence of three flavonols - kaempferol, quercetin and myricetin - in a normal diet is linked with a 23% reduction in the risk of pancreatic cancer, a rare but often fatal disease among smokers (Nothlings *et al.*, 2007).

Table 2. The content of phenolic compounds in the grapevine leaf of the cultivar 'Cardinal' expressed in mg/kg (mean ± standard error, n = 20)

No	Compound	Leaf blade	Petiole	Concentration ratio
1	Procyanidin dimer 1	718.69 ± 30.11	205.36 ± 12.77	3.50
2	Procyanidin dimer 2	736.07 ± 69.31	229.09 ± 9.42	3.21
3	Procyanidin dimer 3	609.14 ± 36.07	274.88 ± 7.16	2.22
4	Procyanidin B1	575.56 ± 41.58	220.17 ± 14.93	2.61
5	Caftaric acid	252.07 ± 20.60	44.79 ± 3.98	5.63
6	*p*-coumaroyl-hexose	25.93 ± 0.90	11.10 ± 0.32	2.34
7	Catechin	169.86 ± 10.42	203.24 ± 16.09	0.84
8	Procyanidin B2	350.92 ± 25.49	81.79 ± 8.68	4.29
9	Derivative of the sinapic acid and glucose	154.60 ± 4.88	46.33 ± 3.00	3.34
10	Coutaric acid	48.54 ± 3.24	20.72 ± 1.07	2.34
11	Epicatechin	263.82 ± 14.59	138.44 ± 5.41	1.91
12	Quercetin-3-*O*-rutinoside	731.59 ± 38.03	110.52 ± 7.57	6.62
13	Quercetin-3-*O*-galactoside	343.08 ± 19.23	34.98 ± 2.45	9.81
14	Quercetin-3-*O*-glucoside	1176.96 ± 66.72	100.49 ± 7.32	11.71
15	Quercetin-3-*O*-glucuronide	3794.82 ± 54.55	387.80 ± 18.54	9.79
16	Kaempferol-3-*O*-rutinoside	123.60 ± 6.25	10.23 ± 0.78	12.08
17	Kaempferol-3-*O*-galactoside	56.78 ± 2.89	3.66 ± 0.23	15.52
18	Kaempferol-3-*O*-glucoside	183.70 ± 11.03	8.62 ± 0.63	21.31
19	Kaempferol-3-*O*-arabinoside	228.83 ± 9.40	13.09 ± 1.07	17.48

a)



b)

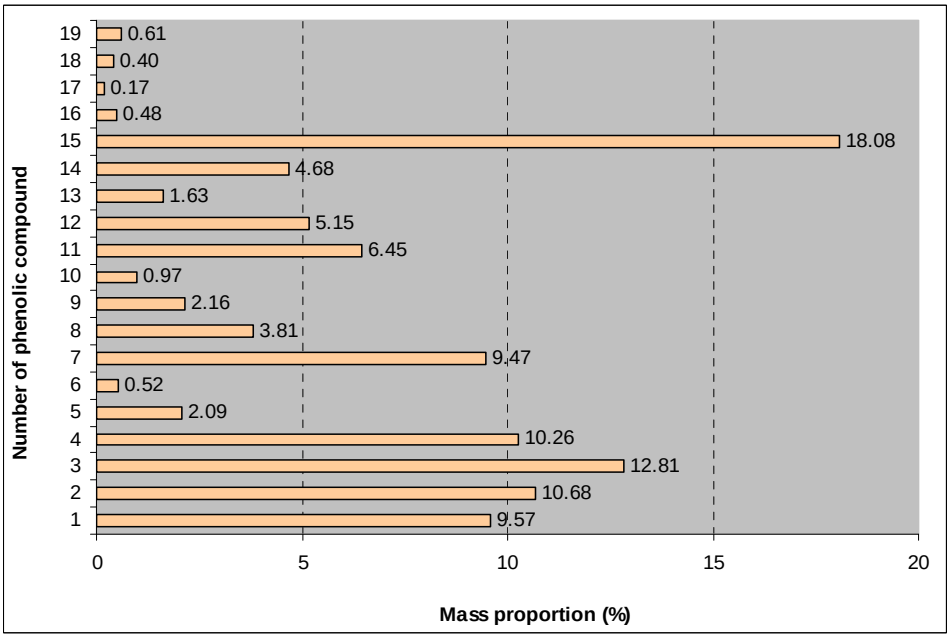


Figure 2. Mass proportion of phenolic compounds in the leaf blade (a) and in the petiole (b). The numbers of the compounds correspond with those stated in Tables 1 and 2 above.

Quercetin and its glycosides are also the most frequent ingredients of plants used against snake bites. Mors *et al.* (2000) established that this compound is a strong inhibitor of lipoxygenase. Moreover, flavonoids are inhibitors of some other enzymes, such as cyclooxygenase, monooxygenase, xanthine oxidase, mitochondrial succinoxidase, NADPH-oxidase, phospholipase A2 and protein kinase (Dugas *et al.*, 2000). Compared with other secondary metabolites, flavonoids are the most capable of creating complex formations due to an abundance of opportunities for forming hydrogen bonds around a relatively small carbon skeleton. The establishment of a hydrogen bond of the phenolic OH group with amides of the protein chain causes them to bind with biological polymers, e.g. enzymes. The effects of flavonoids are usually attributed to their antioxidative properties. The inhibition of enzymes by flavonoids is due to their ability to react with reactive oxygen species, which form on a reactive centre or close to it (Takahama, 1985).

On the other hand, the research team of the Linus Pauling Institute of the US University of Oregon and the European Food Safety Authority (EFSA) has concluded that flavonoids have a very small or no direct antioxidative value in organisms but they increase the production of uric acid, a natural antioxidant that accounts for about half of the total antioxidative capacity of blood plasma. This effect, caused by the excretion of flavonoids, was recorded after consumption of food rich in these compounds (Lotito and Frei, 2006, Williams *et al.*, 2004; EFSA Panel on Dietetic Products, Nutrition and Allergies, 2010).

CONCLUSION

In the leaf of the grapevine cv. ‘Cardinal’, derivatives of hydroxycinnamic acid, flavonols and flavan-3-ols were identified. The leaf blade of the grapevine was significantly richer in phenolic compounds (about five times more, by the sum of concentrations) compared with the petiole. Among the compounds identified, only the content of catechin was higher in the petiole than in the leaf blade. The leaf blade and the petiole had the highest concentration of quercetin-3-glucuronide.

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Ana TOPALOVIĆ, Maja MIKULIČ-PETKOVŠEK, Natalija PEROVIĆ, Snežana TRIFUNOVIĆ and Mirko KNEŽEVIĆ

FENOLNI SASTAV LISTA VINOVE LOZE SORTE ‘KARDINAL’

SAŽETAK

HPLC/DAD/ESI/MS analizom liske i peteljke lista vinove loze sorte ‘Kardinal’ utvrđen je njihov fenolni sastav. Identifikovani su: derivati hidroksicimetnih kiselina – kaftarna i kutarna kiselina, derivati sinapinske kiseline i glukoze, *p*-kumarinske kiseline i heksoze; flavonoli – kvercetin-3-rutinozid, kvercetin-3-galaktozid, kvercetin-3-glukozid, kvercetin-3-glukuronid, kempferol-3-rutinozid, kempferol-3-galaktozid, kempferol-3-glukozid, kempferol-3-arabinozid; i flavan-3-oli – katehin, epikatehin, procijanidin dimeri i procijanidin B1 i B2. Nađeno je da je liska mnogo bogatija fenolnim jedinjenjima u poređenju sa peteljkom. Oba ispitivana dijela lista sadržala su najviše kvercetin-3-glukuronida.

Ključne riječi: liska, peteljka, fenolna jedinjenja