Characterization of the Polyphenolic Composition of Purple Loosestrife (*Lythrum salicaria*)

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Phenolic compounds of purple loosestrife (*Lythrum salicaria* L.) were analysed by the use of liquid chromatography – mass spectrometry (LC/MS) equipped with atmospheric pressure chemical ionisation (APCI) and electrospray ionisation (ESI). The presence of vitexin and orientin as well as their isomers, isovitexin and isoorientin, were confirmed using ion trap multiple stage LC/MS^3 analysis. Several phenolic acids and tannins were also detected. Ellagitannins, vescalagin and pedunculagin, are reported from the plant for the first time.

Introduction

Purple loosestrife (*Lythrum salicaria* L., Lythraceae) is a widely distributed plant in Europe, North America and Asia. It is an agressive invader that grows typically in fens, riversides and other damp places. *Lythrum salicaria* has been the subject of several phytochemical studies during the 1960s and 1970s. It contains anthocyanosides (Paris and Paris, 1964), flavonoid *C*-glycosides and phenolic acids (Paris, 1967; Torrent Marti, 1975) as well as a number of neutral constituents (Fujita *et al.*, 1972). Tannins are present in significant amounts, 6% dry weight in stalks to 24% in leaves (Mihajlovic, 1988), and four new tannins have been reported recently (Ma *et al.*, 1996). Although many species of Lythraceae are rich in alkaloids (Ferris *et al.*, 1966; Fujita *et al.*, 1967), it has been reported that *Lythrum salicaria* does not contain any remarkable amounts of them (Steinfeld, 1969; Fujita *et al.*, 1972). Chemical constituents reported from *L. salicaria* with appropriate references are given in Table I.

Purple loosestrife is traditionally used as a drug, as an antidiarrheic in the form of a decoction or a fluid extract. The flowering top may be used in the treatment of subjective symptoms of venous insufficiency, and the symptoms of haemorrhoids (Bruneton, 1995). Furthermore, extracts of purple loosestrife have been reported to possess hypoglycemic and molluscicidal activity (Cadavid Torres and Calleja Suarez, 1980; Schaufelberger and Hostettmann, 1983). In a large bioactivity screening project of Finnish plant material, a methanolic extract of *L. salicaria* was proved to have both antifungal and calcium antagonistic activity *in vitro* (Rauha *et al.*, 1999; Rauha *et al.*, 2000).

In order to determine the background for results obtained previously, both further biological activity tests and phytochemical data of the extract are needed. Fractionation is necessary to concentrate minor compounds and render their on-line identification unambiguous. In this study, the phytochemical information of *Lythrum salicaria* published in literature was collated. The phenolic composition of the crude extract of this plant was characterised using HPLC/DAD-UV, LC/APCI-MS and LC/ESI-MS techniques.

Materials and Methods

Plant material

Lythrum salicaria L. was collected during the flowering season (July 1998) in Helsinki, and identified by Pia Fyhrqvist (M.Sc.(Bot.)). The plants

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were air dried at room temperature for two weeks, and milled using a grinder.

Extraction

270 g of powdered plant material were loaded into a Büchi 17980 column (460 mm × 49 mm i.d., Büchi Laboratoriums-Technik AG, Flawil, Switzerland) and extracted with 80% aqueous methanol (technical quality, Algol, Espoo, Finland) using a medium pressure solid/liquid extraction (MPSLE) technique according to Mousa (1995). The solvent was pumped through the column at a flow rate of 5 ml/min for 30 min and thereafter stopped for 2 h (Waters M-6000A Chromatography pump, Waters Inc., Milford, MA, USA). This procedure was automatised and repeated 15 times. The extract was concentrated under vacuum and yielded 67 g of material.

Reversed phase chromatography fractionation

The crude extract was fractionated using a Büchi 17982 MPLC column (460 mm × 26 mm i.d.) packed with 145 g of LiChrospher RP-18 stationary phase (particle size 15–25 μm, E. Merck, Darmstadt, Germany). The mobile phase consisted of MeOH and MeCN (HPLC quality, Rathburn, Walkerburn, UK) in the ratio 15:4 v/v (B), water (Milli Q purified) (A). A and B contained both 1% of formic acid (pro analysis, Riedel-de-Haën, Seelze, Germany). 3 g of the crude extract (in 10 ml of 10% B) were separated using a step gradient (flow rate 2.0 ml/min): 0–20 min 10% B, 20–60 min 20% B, 60–90 min 35% B, 90–140 min 60% B, and 140–380 min 90% B (Kontron T-414 LC pump, Tegimenta AG, Rotkreuz, Switzerland). This gradient was designed using the DryLab® method optimisation programme (LC Resources, Walnut Creek, CA, USA). The RP-18 separation yielded 5 fractions: R1 120–174 min (656 mg), R2 174–201 min (165 mg), R3 201–228 min (83 mg), R4 228–264 min (69 mg), and R5 264–327 min (10 mg).

Gel chromatography fractionation

The crude extract was also fractionated with an open column system. 600 ml of Sephadex LH-20 (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) in methanol was packed into an open chromatography column (70 cm × 3.5 cm i.d.). 1 g of crude extract solubilised in 80% MeOH was applied onto the stationary phase. 80% MeOH was eluted at a flow rate of 1 ml/min (for the first 800 ml, and thereafter 100% MeOH) and yielded 9 fractions: S1 180–336 ml (11 mg), S2 337–462 ml (203 mg), S3 463–570 ml (178 mg), S4 571–672 ml (83 mg), S5 673–924 ml (56 mg), S6 925–1026 ml (24 mg), S7 1027–1176 ml (21 mg), S8 1177–1380 ml (32 mg), and S9 1381–1678 ml (24 mg).

HPLC/DAD-UV analysis (Method A)

The HPLC/DAD-UV analysis was carried out using a Hewlett-Packard Series 1100 Pump, Column thermostat (+25 °C) and Autosampler (Hewlett Packard GmbH, Waldbronn, Germany), and Series 1100 degasser and diode array detector (Hewlett Packard, Japan). Spectra were recorded between 190–500 nm, and each chromatogram was detected at 3 different wavelengths 210 nm, 254 nm and 366 nm.

The crude extract and different fractions were analysed on a Symmetry RP-18 HPLC column (4 μm, 250 mm × 4.6 mm i.d., Waters Associates, Milford, MA, USA) with a Guard-Pak C18 (Waters) pre-column. The gradient consisting of MeOH (Super purity solvent, Romil Ltd, Cambridge, UK, 0.05% TFA added) in 0.05% aqueous trifluoroacetic acid (TFA, Uvasol, for spectroscopy, Merck, Darmstadt, Germany) was 0–15 min 4% isocratic, 15–20 min 4–10% linear gradient, 20–25 min 10% isocratic, 25–50 min 10–40% linear gradient, 50–85 min 40–100% linear gradient, and 85–95 min 100% isocratic at a flow rate 1 ml/min.

HPLC/UV/APCI-MS^n analysis of flavonoids (Method B)

The identities of the flavonoid-*C*-glycosides were determined using a modified HPLC/UV/APCI-MS^n method, originally described by Rath *et al.* (1995). The HPLC consisted of Hewlett-Packard Series 1100 pump, autosampler, degasser, column thermostat (+25 °C) and diode array detector. MS data was collected on a Finnigan MAT LCQ ion trap mass spectrometer (San Jose, CA, USA). Positive ions were introduced by atmospheric pressure chemical ionisation (APCI) interface (corona discharge needle 5 μA, vaporizer temperature 450 °C, sheath gas (N_2) flow rate 0.55 MPa, capillary temperature 200 °C). On the ion trap the sequence of three scan events was as follows: a) full scan *m/z* 75–800 (MS^1), b) selective isolation and fragmentation of the protonated molecules at *m/z* 433 and 449 (MS^2), c) selective isolation and fragmentation of the $[M+H-120]^+$ fragments at *m/z* 313 and 329 issued from the MS^2 step (MS^3). For these MS^n steps the energy was set at 50% and the isolation width at 4 Da.

HPLC separations were carried out on a Symmetry RP-18 HPLC column (4 μm, 250 mm × 4.6 mm i.d., Waters Associates, Milford, MA, USA) with a Guard-Pak C18 (Waters) pre-column. An isocratic separation was performed with an MeCN-water (0.05% TFA) 14:86 v/v mixture during 40 min, and finally washed with 100% MeCN (Super purity solvent, Romil Ltd). The LC/UV traces were recorded on-line with detection at 366 nm.

HPLC/UV/ESI-MS analysis of tannins and phenolic acids (Method C)

The HPLC/UV system consisted of two Perkin-Elmer Series 200 micro pumps (Perkin-Elmer, Norwalk, CT, USA), Series 200 autosampler (Perkin-Elmer, Norwalk, CT, USA) and a 785A UV/Vis detector (Perkin-Elmer, Norwalk, CT, USA). Detection was performed at 260 nm. MS data was collected on line using a PE Sciex API 365 triple quadrupole mass spectrometer (Sciex, Toronto, Canada) equipped with Turbo ion spray interface. Negative ions were introduced via this pneumatically assisted electrospray ionisation (ESI) interface (needle voltage –4000 V, heated nitrogen gas temperature 300 °C, orifice plate voltage –35 V, ring voltage –220 V, nebulizer gas at position 9, curtain gas at position 12) and analysed in a mass range of *m/z* 100–2000.

HPLC separations were carried out on a Superspher 100 RP-18 column (75 mm × 4 mm i.d., 4 μm, Merck, Germany) as described by Salminen *et al.* (1999). Gradient of MeCN (Lab-Scan, Dublin, Ireland) in 0.4% aqueous formic acid (FF Chemicals, Yli-Ii, Finland) was 0–3 min, 0% isocratic; 3–30 min, 0–30% (linear gradient); 30–35 min, 30–40% (linear gradient) at a flow rate 1 ml/min.

Results and Discussion

HPLC/DAD-UV (Method A)

The HPLC profile of the crude aqueous methanolic extract of *Lythrum salicaria* was extremely complex, and good resolution of all constituents could not be obtained within a single HPLC run (Fig. 1). In order to characterise and localise precisely in the HPLC chromatogram the different constituents of this plant, two different types of fractionations of the crude extract were performed, one with a C18 MPLC column yielding five fractions (R1–5) and another with an open dextran gel column yielding nine fractions (S1–9). All these enriched fractions were studied by LC/UV and LC/MS for the establishment of the metabolic profile of *L. salicaria*. The gradient **A** (see Materials and Methods) was optimised using the crude extract of *L. salicaria*, in order to separate as many peaks as possible, and to be able to collect UV spectral data for the compounds in all fractions.

The UV spectra of four peaks appearing at retention time 52.8–56.5 min in S5, S6 and R3 (peaks p, q, s, t in Fig. 1), were very similar to those of flavone *C*-glycosides (Rath *et al.*, 1995). Most of the peaks of S7 to S9 and R1, having short retention times (a–l in Fig. 1), produced spectra that fitted with those of ellagitannins described by Salminen *et al.* (1999). The peak at 59.3 min (u) had the same UV pattern as ellagic acid (max 254 and 368 nm, min 328 nm), and was present at a high concentration in S7 and S8. Other phenolic acids were not recognizable based on UV-data only.

Identification of flavone-C-glucosides (Method B)

The flavone-*C*-glucosides composition of the crude extract of *L. salicaria* was investigated by LC/APCI-MS experiment (positive ion mode) using a broad MeCN-water gradient (Method **B**). Peaks producing ions *m/z* 449 and 433, and thus being in accordance with the values expected for orientin and vitexin, as well as their isomers, were detected.

In order to determine the nature of the isomers detected, multiple stage MS^n experiments were performed on line. The protonated molecules of

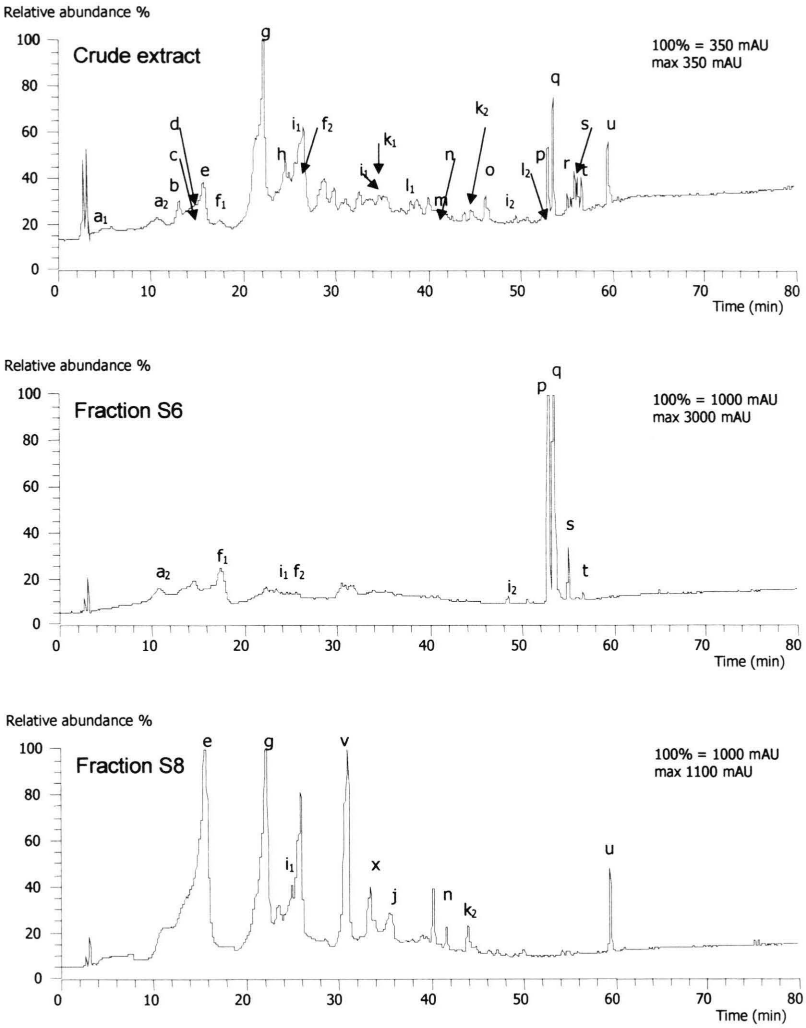

Fig. 1. HPLC/UV trace of crude *Lythrum salicaria* extract and enriched fractions S6 and S8 detected at wavelength 210 nm using method **A**. Attribution of the peaks: See Table I; v and x, methylated vescalagin and castalagin.

interest were isolated in the ion trap mass analyser, selectively excited, and their fragments analysed (MS^2). This step was repeated on fragments, generating MS^3 spectra. It has been shown that under these conditions, a clear difference could be made between isomeric pairs of *C*-glycosyl flavones (Rath *et al.*, 1995).

The enriched fraction R3 was analysed by LC/APCI/MS^3 under isocratic conditions. On the single ion traces, two peaks (p, q) with protonated molecules at *m/z* 449 were recorded at 17.4 and 20.2 min, and two peaks (s, t) with $[M+H]^+$ ions at *m/z* 433 at 30.8 and 33.5 min, respectively (Table I). The MS^2 spectra of the $[M+H]^+$ ions p and q were similar, showing losses of 90, 120 and 150 Da characteristic for flavonoid-*C*-glucosides (Rong *et al.*, 1998). However, the MS^3 spectra of the $[M+H-120]^+$ ions obtained in MS^2 of these isomers revealed clear differences. The MS^3 of peak p produced major ions at *m/z* 311, 300 and 283 characteristic of losses of 18 (-H_2O), 29 (-CHO) and 46 (-CO, -H_2O) Da, while the MS^3 of peak q only showed a major loss of 29 Da. In comparison to results already obtained on known standards, the MS^3 spectrum of p was characteristic for a flavone-*C*-glycoside substituted in position 6, while

Table I. Phenolic compounds detected in *Lythrum salicaria* in current study and all constituents reported in literature from *L. salicaria.*

			Data obtained in current study							
Class	Compound	MW	M+H	M-H	Other *m/z* values	Peaks	t_R (min)	Method	Isomers	Reference
Tannins	HHDP-glucose	482		481	301	a_1, a_2	1.8; 2.8	C	2	d.f.
	1-*O*-galloylglucose	332		331	169	c	4.8	C		d.f.
	6-*O*-galloylglucose	332		331	169	d	7.3	C		d.f.
	Vescalagin	934		933	1400, 466, 1867, 631, 301	e	8.2	C		d.f.
	Pedunculagin	784		783	391	f_1, f_2	9.5; 12.0	C		d.f.
	Castalagin	934		933	1400, 466, 1867, 631, 301	g	10.5	C		Ma *et al.* (1996)
	1,6-di-*O*-galloylglucose	484		483		n	14.2	C		d.f.
	Galloyl-HHDP-glucose	634		633	316	j	14.8	C	1	d.f.
	Trigalloylglucose	636		635		k_1, k_2	14.7; 17.6	C	2	d.f.
	Galloyl-bis-HHDP-glucose	936		935		l_1, l_2	15.2; 18.5	C	2	d.f.
	Trigalloyl-HHDP-glucose	938		937		r	19.7	C	1	d.f.
	Lythrine A	1868								Ma *et al.* (1996)
	Lythrine B	1868								Ma *et al.* (1996)
	Lythrine C	1820								Ma *et al.* (1996)
	Lythrine D	1868								Ma *et al.* (1996)
Flavonoids	Isoorientin	448	449		431, 413, 395, 329, 299, 359	p	17.4	B		Paris (1966)
	Orientin	448	449		431, 329, 359, 413, 395	q	20.2	B		Paris (1966)
	Vitexin	432	433		313, 343, 415, 397	s	30.8	B		Paris (1966)
	Isovitexin	432	433		313, 415, 379, 343, 283	t	33.5	B		Paris (1966)
	Cyanidin-3-galactoside	449								Paris (1964)
	Malvidin-3,5-diglucoside	655								Paris (1964)
Phenolics	Gallic acid	170		169	125	b	4.6	C		Paris (1966)
	Methyl gallate	184		183		h	11.9	C		Paris (1966)
	Valoneic acid dilactone	470		469	425, 939, 301	i_1, i_2	11.9; 17.3	C	2	d.f.
	Chlorogenic acid	354		353	191, 707	m	14.2	C		Paris (1966)
	Isochlorogenic acid	354		353	191	o	15.7	C		Torrent Marti (1975)
	Ellagic acid	302		301	603, 905, 1207, 1509	u	21.0	C		Paris (1966)
	Caffeic acid	180								Torrent Marti (1975)
	p-Coumaric acid	164								Torrent Marti (1975)
Phthalates	Dibutyl phthalate	278								Fujita *et al.* (1972)
	Diisobutyl phthalate	278								Fujita *et al.* (1972)
	Diisoheptyl phthalate	362								Fujita *et al.* (1972)
	Diisooctyl phthalate	390								Fujita *et al.* (1972)
	Butyl-2-methylpropyl phthalate	278								Fujita *et al.* (1972)
	Phthalic acid	166								Fujita *et al.* (1972)
Sterols	*β*-Sitosterol	414								Fujita *et al.* (1972)
Terpenes	Loliolide	196								Fujita *et al.* (1972)

MW = nominal molecular weight
M+H = protonated molecule
M-H = deprotonated molecule
Peaks = peaks in reference to chromatogram in Fig. 1
t_R = retention time of the compound produced by the method given in column "Method"
Isomers = the number of detected isomers
B = LC/MS method B
C = LC/MS method C
d.f. = detected first time in *L. salicaria*

the MS^3 spectrum of q corresponded to its isomer substituted in position 8 (Fig. 2). Thus peaks p and q were identified as isoorientin and orientin, respectively. The flavonoids s and t were identified as vitexin and isovitexin in a similar way. These flavone-*C*-glucosides were already detected in *L. salicaria* (Paris, 1967; Mihajlovic, 1988).

Identification of tannins and phenolic acids (Method C)

In order to assess the composition of tannins and phenolic acids, LC/ESI-MS in the negative ion mode was used (Method **C**). Molecular weight assignment was based on the strong $[M-H]^-$ ion obtained, as well as on the presence of respective

Compound	R_1	R_2	R_3
Isoorientin	OH	Glu	H
Orientin	OH	H	Glu
Vitexin	H	H	Glu
Isovitexin	H	Glu	H

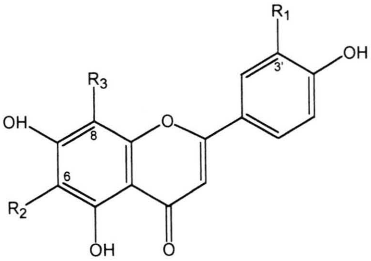

Fig. 2. Structures of flavonoid-*C*-glucosides of *Lythrum salicaria*. Glu = glucose.

$[M-2H]^{2-}$ and $[2M-H]^-$ ions. In this mode, almost no fragmentation was observed. The sensitivity of the ESI detection was much better in negative mode than in positive mode (Salminen *et al.*, 1999).

The display of the single ion traces of the $[M-H]^-$ ion of the peak for gallic acid (t_R = 4.6 min; peak b in Fig. 1), methyl gallate (11.9 min; h), chlorogenic acid (14.2 min; m), and isochlorogenic acid (15.7 min; o), allowed their specific localisation (Table I). However, these compounds were not resolved in the UV- or total ion chromatogram (TIC) traces of the crude extract. In the case of ellagic acid (MW 302; t_R = 21.0 min; u) additional peaks at $[3M-H]^-$ (905), $[4M-H]^-$ (1207) and $[5M-H]^-$ (1509) were also observed. The presence of caffeic-, *p*-coumaric- and phthalic acid could not be confirmed in this study.

Four new tannins, lythrine C (MW 1820) and lythrines A,B,D (MW 1868), were recently identified from *L. salicaria* by a Chinese group (Ma *et al.*, 1996). These compounds could not be detected in our study. Within the spectra of two major tannin peaks (8.2 min and 10.5 min) signal at *m/z* 1867 together with ions at 933 and 466 could have been theoretically attributed to one of the lythrines. However, 1867 corresponded in fact to a dimer of castalagin (g; MW 934). Indeed, in the same spectrum, the main peak at *m/z* 933 represented the $[M-H]^-$ ion of castalagin. The single charge of this ion was verified by the mass difference between the isotopes (1 Da). Another peak at *m/z* 466, its isotopic peak differing by 0.5 Da, fitted with $[M-2H]^{2-}$. Castalagin was reported from *L. salicaria* by Ma *et al.* (1996); however, our results indicated also the presence of its isomer, vescalagin (e) (Fig. 3). Their elution order was reported by Lei *et al.* (1999). Puech *et al.* (1999) reported also ethoxylation of vescalagin and castalagin when stored in aqueous ethanol solution, leading to ketal and hemiketal structures. Our samples were diluted in methanolic solution, and methoxylated castalagin and vescalagin derivatives were detected at *m/z* 963 (13.2 and 14.2 min). The presence of two peaks also supported our hypothesis of the existence of both castalagin and vescalagin in *L. salicaria*. When using the method **A**, the retention times of vescalagin and castalagin were 15.6 min and 22.1 min, respectively (Fig. 1), and their UV spectra were identical and concordant with a spectrum of ellagitannins without any free galloyl group.

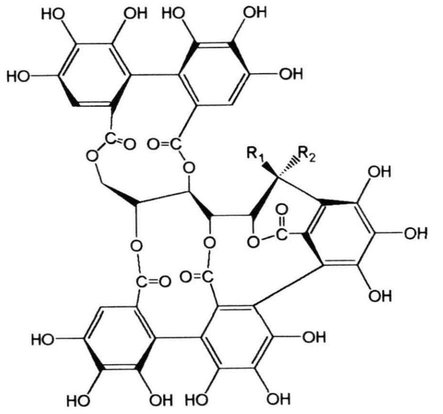

Fig. 3. Structure of vescalagin (R_1 = OH, R_2 = H) and castalagin (R_1 = H, R_2 = OH).

The study of the single ion traces of the crude extract using method **C** revealed a peak at 4.8 min displaying a $[M-H]^-$ ion *m/z* 331. This could be attributed most probably to 1-*O*-galloylglucose (peak c). The presence of a gallic acid fragment at *m/z* 169 confirmed this attribution. The position of the galloyl group at C-1 was supported by the biosynthetic pathway (Haslam, 1998). The identification of valoneic acid dilactone isomers (peaks i_1, i_2) was based on ions corresponding to $[M-H]^-$ (469), to $[M-COOH]^-$ (425), to $[2M-H]^-$ (939), and to $[M-170-H]^-$ (301), the fragmentation of which could be found also in literature (Nawwar *et al.*, 1997). The peak of 1,6-di-*O*-galloylglucose (n) and the two peaks of pedunculagin, i.e. bis-HHDP-glucopyranose (f_1, f_2), were identified by comparing the current data with UV- and MS spectra and retention times of hydrolysable tannins recently isolated from birch (*Betula pubescens* Ehrh., Betulaceae) leaves and structurally determined as 1,6-digalloylglucose and pedunculagin using ^{1}H- and ^{13}C-NMR data (Salminen, unpublished results). The two peaks of pedunculagin resulted from the presence of α- and β-glucose isomers.

For the rest of the compounds, only partial structure assignment was performed based on LC/MS data. Information on the nature of sugars and linkage positions of galloyl or hexahydroxydiphenoyl (HHDP) could not be ascertained. A deeper structure investigation would require the isolation of all compounds of interest.

The phytochemical data obtained from the aerial part of *Lythrum salicaria* were collated from the literature and for the most part confirmed in this study. The combined results of this series of analyses are presented in Table I. All the identified compounds have been indicated in the HPLC/DAD-UV chromatogram from the analysis of the crude extract using the combined chromatographic and spectral data (Fig. 1). No evidence of lythrines was detected in our Finnish plant material, but ellagitannins, vescalagin and pedunculagin, are reported from this plant for the first time.

Acknowledgements

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Bruneton J. (1995), Pharmacognosy, Phytochemistry, Medicinal Plants. Lavoisier Publishing, Hampshire.

Cadavid Torres I. and Calleja Suarez J. (1980), A preliminary study of hypoglycemic activity of *Lythrum salicaria.* J. Nat. Prod. **43**, 559–563.

Ferris J. P., Boyce C. B., Briner R. C., Douglas B., Kirkpatrick J. L. and Weisbach J. A. (1966), Lythraceae alkaloids. Structure and stereochemistry of the major alkaloids of Decodon and Heimia. Tetrahedron Lett. **30**, 3641–3649.

Fujita E., Fuji K., Bessho K., Sumi A and Nakamura S. (1967), The structures of lythranine, lythranidine and lythramine, novel alkaloids from *Lythrum anceps* Makino. Tetrahedron Lett. **46**, 4595–4600.

Fujita E., Saeki Y., Ochiai M. and Inoue T. (1972), Investigation of the neutral constituents of *Lythrum salicaria.* Bull. Inst. Chem. Res., Kyoto Univ. **50**, 327–331.

Haslam E. (1998), Practical Polyphenolics. From Structure to Molecular Recognition and Physiological Action. Cambridge University Press, Cambridge, UK.

Lei Z. T., Jervis J. and Helm R. F. (1999), *C*-glycosidic ellagitannins from white oak heartwood and callus tissues. Phytochemistry **51**, 751–756.

Ma X., Ji C., Wang Y., Zhang G. and Liu Y. (1996), New tannins from *Lythrum salicaria* L. J. Chin. Pharm. Sci. **5**, 225.

Mihajlovic N. (1988), Uporedno farmakognozijsko proucavanje biljaka *Lythrum salicaria* L. i *Lythrum virgatum* L., Lythraceae. Arh. Farm. **38**, 29–35.

Mousa O. (1995), Ethnopharmacological and phytochemical studies on certain Egyptian *Ficus* species. Dissertation book. Cosmoprint Oy, Helsinki.

Nawwar M. A. M., Marzouk M. S., Nigge W. and Linscheid M. (1997), High-performance liquid chromatographic/electrospray ionization mass spectrometric screening for polyphenolic compounds of *Epilobium hirsutum* – The structure of the unique ellagitannin Epilobamide-A. J. Mass Spectrom. **32**, 645–654.

Paris R. R. and Paris M. (1964), Sur les pigments anthocyaniques de la Salicaire (*Lythrum salicaria* L.). Compt. Rend. **258**, 361–364.

Paris M. (1967), Contribution à l'étude biochimique de la Salicaire (*Lythrum salicaria* L., Lythracées) et en particulier de ses polyphenols. Trav. Lab. Matiere Med. Pharm. Galenique Fac. Pharm. Paris **52**, I/1–111.

Puech J. L., Mertz C., Michon V., le Guernevé C., Doco T. and Hervé du Penhoat C. (1999), Evolution of castalagin and vescalagin in ethanol solutions. Identification of new derivatives. J. Agric. Food Chem. **47**, 2060–2066.

Rath G., Touré A., Nianga M., Wolfender J.-L. and Hostettmann K. (1995), Characterization of *C*-glycosylflavones from *Dissotis rotundifolia* by liquid chromatography – UV diode array detection – tandem mass spectrometry. Chromatographia **41**, 332–342.

Rauha J.-P., Tammela P., Summanen J., Vuorela P., Kähkönen M., Heinonen M., Hopia A., Kujala T., Pihlaja K., Törnquist K. and Vuorela H. (1999), Actions of some plant extracts containing flavonoids and other phenolic compounds on calcium fluxes in clonal rat pituitary GH_4C_1 cells. Pharm. Pharmacol. Lett. **9**, 66–69.

Rauha J.-P., Remes S., Heinonen M., Hopia A., Kähkönen M., Kujala T., Pihlaja K., Vuorela H. and Vuorela P. (2000), Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int. J. Food Microbiol. **56**, 3–12.

Rong H., Stevens J. F., Deinzer M. L., de Cooman L. and de Keukeleire D. (1998), Identification of isoflavones in the roots of *Pueraria lobata*. Planta Med. **64**, 620–627.

Salminen J.-P., Ossipov V., Loponen J., Haukioja E. and Pihlaja K. (1999), Characterisation of hydrolysable tannins from leaves of *Betula pubescens* by high-performance liquid chromatography – mass spectrometry. J. Chromatogr. A **864**, 283–291.

Schaufelberger D. and Hostettmann K. (1983), On the molluscicidal activity of tannin containing plants. Planta Med. **48**, 105–107.

Steinfeld A. S. (1969), The alkaloids of *Lythrum salicaria*. The electrolytic oxidation of some phenolic tetrahydroisoquinolines. Diss. Abstr. B **29**, 2820.

Torrent Marti M. T. (1975), Estudio farmacognóstico y farmacodinámico de *Lythrum salicaria* L. Circ. Farm. **33**, 265–307.