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THE STILBENE AND CATECHIN CONTENT OF THE SPRING SPROUTS OF *REYNOUTRIA* SPECIES ◊

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SUMMARY

Knotweed rhizomes and young sprouts are used in a traditional Asian medicine. Because knotweed rhizomes contain large amount of stilbenes and catechins, the main objective of this study was analysis of selected phenolic compounds, including stilbenes and catechins, in young spring sprouts of the knotweed genus (Japanese knotweed (*Reynoutria japonica*), Giant knotweed (*Reynoutria sachalinensis*), and their hybrid Bohemian knotweed (*Reynoutria* × *bohemica*)). Plant material was collected in the Czech Republic. Dry sprouts were extracted with 90% methanol and phenolic compounds were analysed by HPLC–DAD. The sprouts of all three species contain large amounts of resveratrol, piceid, catechin, and epicatechin but the total quantity of these phenolic compounds was less than in the rhizomes.

INTRODUCTION

The knotweeds (*Reynoutria* Houtt. genus) have spread around Europe, North America, and New Zealand as decorative plants introduced from East Asia [1–5]. They are extremely persistent invasive plants [6] growing along roads and waterways, and on dumps and abandoned or degraded fields [7]. There are three species of knotweed – Japanese knotweed, *Reynoutria japonica* (Houtt.), giant knotweed, *Reynoutria sachalinensis* (F. Schmidt) Nakai, and their hybrid Bohemian knotweed, *Reynoutria* × *bohemica* (Chrtek et Chrtková), all in the *Polygonaceae* family [8].

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Knotweed plants are interesting because of the relatively high concentrations of biologically active substances they contain. They have, therefore, been the objects of two types of investigation. The first is study of the rhizomes. Resveratrol, piceid, and other stilbenes have been detected in the rhizomes of Japanese knotweed [9–13], as have several other biologically active substances with estrogenic activity, for example emodin and other derivatives of 9,10-anthraquinone [11,12,14,15]. Emodin, physcion, and glycosides of 9,10-anthraquinone derivatives have been detected both in the rhizomes and in the aerial part of giant knotweed; these compounds have allelopathic properties [16]. The second type of investigation has been directed especially at substances with fungicidal effects present in leaves of the giant knotweed [17,18].

Since 2003 our team has been interested in the detection of specific compounds in all three knotweed species [13,19,20]. Knotweed rhizomes are used in a traditional Asian medicine, as also are knotweed young sprouts. The main objective of this study was analysis of selected phenolic compounds (including stilbenes and catechins) in the young spring sprouts.

EXPERIMENTAL

Spring sprouts of knotweed were collected in April 2003, in the Český Krumlov district of the Czech Republic, from five specimens of each species (*R. japonica*, *R. sachalinensis*, and *R.* × *bohemica*). The spring sprouts were dried at laboratory temperature and the material was then pulverized and extracted with 90% methanol. Each sample was prepared and analysed separately.

The extracts were analysed with a Hewlett–Packard (HP) 1050 HPLC with an HP 1040A diode-array detector (DAD). Compounds were separated on a 2 mm × 150 mm, 3-µm particle, Phenomenex Luna C_{18} column. The mobile phase, a water–acetonitrile gradient containing orthophosphoric acid, was prepared from 5% acetonitrile in water containing 0.1% orthophosphoric acid (component A) and 80% acetonitrile in water containing 0.1% orthophosphoric acid (component B). The gradient was from 2 to 50% B in 55 min then from 50% to 90% B in 65 min. The mobile phase flow rate was 0.25 mL min^{-1}, the volume injected 5 µL, and the analysis temperature 25°C. Stilbenes (resveratrol and its derivatives) were detected at 315 nm and catechins at 220 nm. Spectra were recorded in the range 190 to 600 nm.

The amounts of resveratrol and peaks 1 and 3 in the extracts (Figs

1 and 2) were calculated by use of a calibration plot prepared for resveratrol, the amount of piceid was calculated by use of a calibration plot prepared for piceid, and the amounts of catechin and epicatechin were calculated by use of calibration plots prepared for catechin and epicatechin, respectively. Catechin, epicatechin, and resveratrol standards were obtained from Sigma–Aldrich, Czech Republic, and piceid standard from ChromaDex, USA.

Data for the phenolic compound content of the samples were analysed by analysis of variance (Anova) using Statistica software.

RESULTS AND DISCUSSION

We found catechin, epicatechin, piceid, resveratrol, and two resveratrol derivatives (peaks 1 and 3) in young spring sprouts from all three species of knotweed (Figs 1 and 2; Table I). Young spring sprouts are similar to the rhizomes of knotweed with regard to both qualitative and quantitative composition. The dominant compounds in the aerial parts of the knotweed plants are flavonols [19].

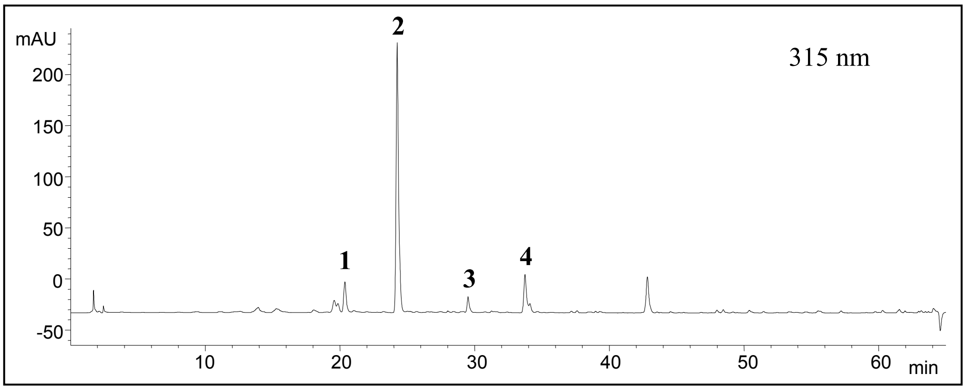

Fig. 1

Chromatogram of phenolic compounds from spring sprouts of Japanese knotweed (*Reynoutria japonica*). Peaks: 1, resveratroloside; 2, piceid; 3, unidentified derivative of resveratrol; 4, resveratrol (detection at 315 nm)

Piceid was the dominant stilbene derivative in all knotweed sprouts monitored; the smallest amount was found in *R.* × *bohemica* (Table I). The resveratrol content of the sprouts of all three types of knotweed was similar (Table I) but although the amount was not negligible, extracts of the rhizo-

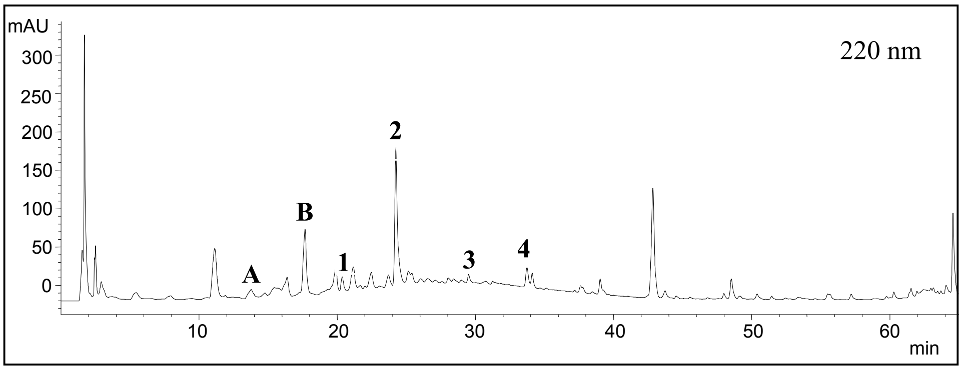

Fig. 2

Chromatogram of phenolic compounds from spring sprouts of Giant knotweed (*Reyno-utria sachalinensis*). Peaks: A, catechin; B, epicatechin; 1, resveratroloside (see text); 2, piceid; 3, unrecognised derivative of resveratrol; 4, resveratrol (detection at 220 nm)

Table I

Catechin and stilbene content of young spring sprouts of knotweed species (mg kg^{-1} dry mass)

Species		Catechins		Stilbenes			
		Catechin	Epi-catechin	Resvera-troloside	Piceid	Peak 3	Resvera-trol
R. japonica	Mean	103	568	48	683	17	64
	RSD[a]	54	39	87	42	50	101
R. × *bohemica*	Mean	41	230	11	215	18	23
	RSD	45	50	33	48	45	68
R. sachalinensis	Mean	167	674	31	502	65	29
	RSD	116	77	154	64	92	68

[a]Relative standard deviation

mes of *R. japonica* contained many times more resveratrol than the sprouts [9–13]. The rhizomes of *R. japonica* are the largest known source of resveratrol. Peak 3 has a spectrum similar to that of resveratrol, but the identity of this derivative is unknown. The UV spectra of resveratrol and the unidentified derivative are shown in Figs 3 and 4. The spectra were acquired as the compounds eluted; the chromatographic data are given in Table II. On the basis of literature data [9] (comparison of retention times and spectra) we suppose that peak 1 (Fig. 1, Table II) is that of resveratro-loside.

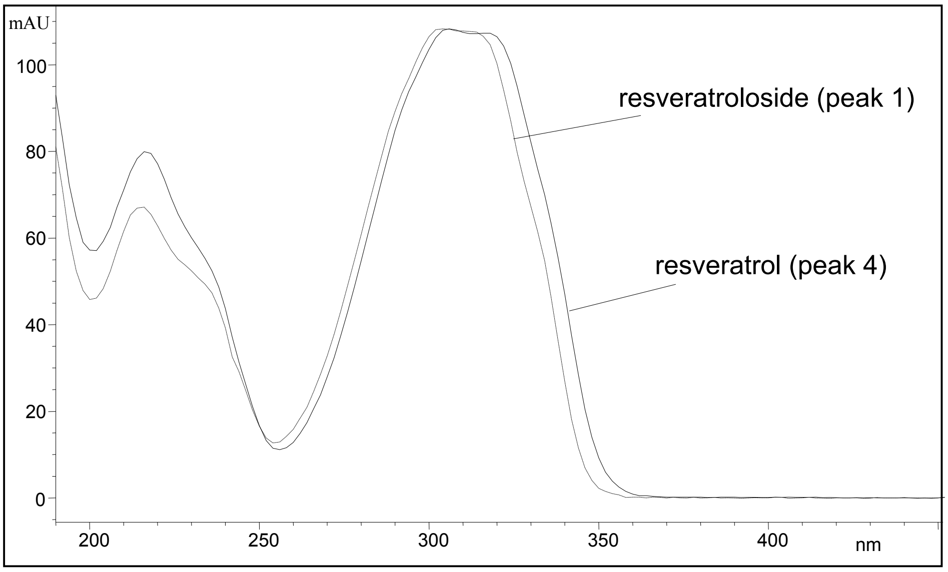

Fig. 3

UV spectra of resveratroloside and resveratrol (peaks 1 and 4 in Figs 1 and 2)

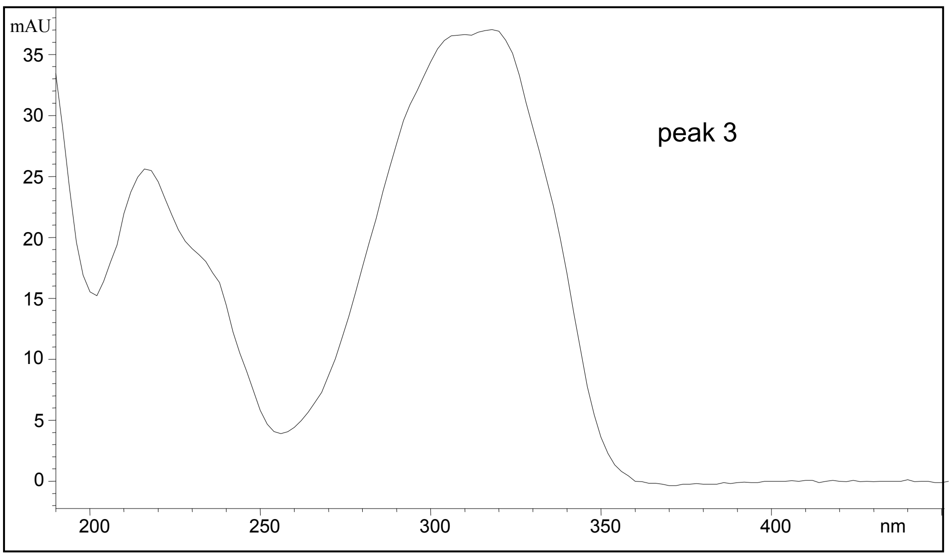

Fig. 4

UV spectrum of unrecognised derivative of resveratrol (peak 3 in Figs 1 and 2)

Table II

Chromatographic data of stilbenes from the chromatogram in Fig. 1

Peak	Stilbene	t_R (min)	Acetonitrile (%)
1	Resveratroloside	20.14	19.69
2	Piceid	24.02	22.22
3	Unrecognized	29.26	25.65
4	Resveratrol	33.51	28.43

The second group of polyphenolic compounds investigated was the catechins (Fig. 2). The amounts of catechin and epicatechin in the hybrid species were less than in the parents (Table I). The catechin content of knotweeds is lower in the sprouts than in the rhizomes but there is no difference between the epicatechin content of the sprouts and rhizomes [13]. The catechin content of the rhizomes of *R.* × *bohemica* is not known.

By statistical analysis of the data we discovered significant differences among the piceid content of the different knotweed species (Anova, $F = 4.30$, $P < 0.05$). There were no significant differences among the amounts of the other polyphenolic compounds in the three knotweed species. The high values of the relative standard deviation (*RSD*) are most probably because the sprouts were of different age.

Knotweed species are important source of stilbenes; in particular, the rhizomes of *R. japonica* contain much more resveratrol than fruit or vegetables [21], grapes [22–24], or wine [25,26]. Although the rhizomes contain more stilbenes than the spring sprouts, young sprouts are probably better a source of these stilbenes in food than are fruit or vegetables.

Stilbenes and catechins are probably important agents in the invasive strategy of the knotweeds. We suppose that these phenolic compounds increase the resistance of the plants to different negative effects of the environment.

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