

Quality Assessment of Japanese Knotweed (*Fallopia japonica*) Grown on Prince Edward Island as a Source of Resveratrol

Huaguo Chen,^{†,§} Tina Tuck,[†] Xiuhong Ji,[†] Xin Zhou,[§] Glen Kelly,[#] Alain Cuerrier,[⊥] and Junzeng Zhang^{*,†}

[†]Aquatic and Crop Resource Development, National Research Council Canada, 550 University Avenue, Charlottetown, PE C1A 4P3, Canada

[§]The Research Center for Quality Control of Natural Medicine, Guizhou Normal University, 116 Baoshan North Road, Guiyang, Guizhou 550001, People's Republic of China

[#]Northeast Biological Processors Ltd., R.R. 3, Charlottetown, PE C1A 7J7, Canada

[⊥]Jardin botanique de Montréal, Institut de recherche en biologie végétale, Université de Montréal, 4101 rue Sherbrooke Est, Montréal, Québec H1X 2B2, Canada

ABSTRACT: Japanese knotweed (*Fallopia japonica*, also known as *Polygonum cuspidatum*) is a common invasive plant species on Prince Edward Island (PEI), Canada, whereas it has been used in Chinese medicine and more recently as a raw material for extracting resveratrol. This paper reports on the quantification of resveratrol, polydatin, emodin, and physcion in roots, stems, and leaves of Japanese knotweed samples from PEI and British Columbia (BC), Canada, and nine provinces of China, by ultraperformance liquid chromatography (UPLC). The results showed that the root contains a much higher level of resveratrol than the stem and leaf, and it is accumulated in its highest level in October. PEI-grown knotweed contains similar levels of resveratrol and polydatin compared to Chinese samples collected in the month of October, but the contents of the other anthraquinones (emodin and physcion) are different. As such, Japanese knotweed grown in PEI could be a commercially viable source of raw material for resveratrol production; however, caution has to be taken in harvesting the right plant species.

KEYWORDS: Japanese knotweed, *Fallopia japonica*, UPLC, resveratrol, quality assessment

■ INTRODUCTION

Fallopia japonica (Houtt.) Ronse Decraene (previously known as *Polygonum cuspidatum* Sieb. & Zucc.), a well-known Chinese herb and officially listed in the Chinese Pharmacopoeia, has been traditionally used for treatment of various inflammatory diseases, hepatitis, tumors, and diarrhea in Eastern Asian countries such as China, Korea, and Japan.^{1–6} In North America, the young stem of *F. japonica* (Japanese knotweed) is edible and was consumed as a vegetable in the past.⁷ Japanese knotweed is listed as one of the “World’s Worst” invaders, and the farming and landscaping sectors have to control its growth and invasive nature.⁸

Over the past decades, the chemical composition^{9–11} and biological activity^{4,5,12,13} as well as quality control^{2,9} of *F. japonica* have been widely reported, and resveratrol, polydatin, emodin, physcion, chrysophanol, rhein, and emodin-8-*O*- β -D-glucoside were revealed as some of the main constituents.

Resveratrol, or *trans*-3,5,4'-trihydroxystilbene, is a naturally occurring antioxidant compound typically associated with grapes and red wine^{14,15} and is also present in *F. japonica*.¹⁶ Extensive research on resveratrol over the past decade has demonstrated significant beneficial effects including the promotion of heart health, neuroprotection, anti-inflammation, and anticancer and antiaging effects, as well as diabetes and obesity prevention.^{17–20} These documented health benefits have raised the profile and interest in resveratrol as an active ingredient in the growing nutraceutical and cosmeceutical industries.

Due to the relatively low level of resveratrol present in grape skin,^{14,21} other plants have been investigated as potential and

commercially viable natural sources of resveratrol for the growing nutraceutical market. The rhizome or root of *F. japonica*, also known as Japanese knotweed, is currently a major source for the natural resveratrol ingredients sold in dietary supplement markets worldwide.^{22,23} On Prince Edward Island (PEI), and other places in Canada, Japanese knotweed is a common invasive plant species. However, until now no information was available on the level of resveratrol in knotweed grown in PEI.

As the demand for natural resveratrol is expected to grow rapidly in the future with increasing applications in the nutraceutical, therapeutic, and cosmeceutical markets, assessing PEI-grown knotweed as a commercial source could present new market opportunities for agriculture and bioresource sectors in the region. In this study, more than 50 knotweed samples collected from 4 different sites from PEI over various seasons have been analyzed for their levels of resveratrol and other components. Knotweed samples from China, which have been used as raw material for resveratrol production, were also studied in parallel.

■ MATERIALS AND METHODS

Instrumentation and Reagents. UPLC analysis was carried out on an Agilent 1200 liquid chromatography system, equipped with a vacuum

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Table 1. *Fallopia japonica* and Other Knotweed Samples Analyzed in This Study

sample	NRC-INH code	Latin name	origin/collection site	acquisition/collection time	plant part
1	INH-OS-114	<i>Fallopia japonica</i>	Zunyi, Guizhou Province, China	Jan 2009	root
2	INH-OS-115	<i>Fallopia japonica</i>	Bijie, Guizhou Province, China	March 2009	root
3	INH-OS-116	<i>Fallopia japonica</i>	Leishan, Guizhou Province, China	June 2009	root
4	INH-OS-117	<i>Fallopia japonica</i>	Dafang, Guizhou Province, China	Aug 2009	root
5	INH-OS-118	<i>Fallopia japonica</i>	Anshun, Guizhou Province, China	Jan 2009	root
6	INH-OS-119	<i>Fallopia japonica</i>	Guiyang, Guizhou Province, China	April 2009	root
7	INH-OS-120	<i>Fallopia japonica</i>	Zhejiang Province, China	Nov 2010	root
8	INH-OS-121	<i>Fallopia japonica</i>	Fujian Province, China	Nov 2010	root
9	INH-OS-122	<i>Fallopia japonica</i>	Sichuan Province, China	Nov 2010	root
10	INH-OS-123	<i>Fallopia japonica</i>	Yunnan Province, China	Nov 2010	root
11	INH-OS-124	<i>Fallopia japonica</i>	Henan Province, China	Nov 2010	root
12	INH-OS-125	<i>Fallopia japonica</i>	Jiangxi Province, China	Nov 2010	root
13	INH-OS-126	<i>Fallopia japonica</i>	Jiangsu Province, China	Nov 2010	root
14	INH-OS-127	<i>Fallopia japonica</i>	Hubei Province, China	Nov 2010	root
15	INH-OS-135	<i>Fallopia × bohemica</i>	St. Margaret's, PEI, Canada	May 14, 2010	root
16	INH-OS-136	<i>Fallopia japonica</i>	Dunstaffnage, PEI, Canada	May 5, 2010	root
17	INH-OS-137	<i>Fallopia japonica</i>	Dunstaffnage, PEI, Canada	May 5, 2010	stem and leaf
18	INH-OS-138	<i>Fallopia × bohemica</i>	St. Margaret's, PEI, Canada	May 20, 2010	root
19	INH-OS-139	<i>Fallopia japonica</i>	Dunstaffnage, PEI, Canada	May 20, 2010	root
20	INH-OS-140	<i>Fallopia japonica</i>	Dunstaffnage, PEI, Canada	May 20, 2010	stem and leaf
21	INH-OS-141	<i>Fallopia × bohemica</i>	St. Margaret's, PEI, Canada	May 20, 2010	stem and leaf
22	INH-OS-142	<i>Fallopia × bohemica</i>	site 1, Summerside, PEI, Canada	May 20, 2010	root
23	INH-OS-143	<i>Fallopia × bohemica</i>	site 1, Summerside, PEI, Canada	May 20, 2010	stem and leaf
24	INH-OS-144	<i>Fallopia × bohemica</i>	St. Margaret's, PEI, Canada	July 13, 2010	stem
25	INH-OS-145	<i>Fallopia japonica</i>	Dunstaffnage, PEI, Canada	July 13, 2010	leaf
26	INH-OS-146	<i>Fallopia × bohemica</i>	St. Margaret's, PEI, Canada	July 13, 2010	leaf
27	INH-OS-147	<i>Fallopia japonica</i>	Dunstaffnage, PEI, Canada	July 13, 2010	root
28	INH-OS-148	<i>Fallopia × bohemica</i>	St. Margaret's, PEI, Canada	July 13, 2010	root
29	INH-OS-149	<i>Fallopia japonica</i>	Dunstaffnage, PEI, Canada	July 13, 2010	stem
30	INH-OS-150	<i>Fallopia × bohemica</i>	site 1, Summerside, PEI, Canada	July 13, 2010	stem
31	INH-OS-151	<i>Fallopia × bohemica</i>	site 1, Summerside, PEI, Canada	July 13, 2010	root
32	INH-OS-152	<i>Fallopia × bohemica</i>	site 1, Summerside, PEI, Canada	July 13, 2010	leaf
33	INH-OS-153	<i>Fallopia × bohemica</i>	St. Margaret's, PEI, Canada	Aug 28, 2010	stem
34	INH-OS-154	<i>Fallopia × bohemica</i>	St. Margaret's, PEI, Canada	Aug 28, 2010	leaf
35	INH-OS-155	<i>Fallopia × bohemica</i>	St. Margaret's, PEI, Canada	Aug 28, 2010	root
36	INH-OS-156	<i>Fallopia japonica</i>	Dunstaffnage, PEI, Canada	Aug 28, 2010	stem
37	INH-OS-157	<i>Fallopia japonica</i>	Dunstaffnage, PEI, Canada	Aug 28, 2010	leaf
38	INH-OS-158	<i>Fallopia japonica</i>	Dunstaffnage, PEI, Canada	Aug 28, 2010	root
39	INH-OS-159	<i>Fallopia × bohemica</i>	site 1, Summerside, PEI, Canada	Aug 28, 2010	stem
40	INH-OS-160	<i>Fallopia × bohemica</i>	site 1, Summerside, PEI, Canada	Aug 28, 2010	leaf
41	INH-OS-161	<i>Fallopia × bohemica</i>	site 1, Summerside, PEI, Canada	Aug 28, 2010	root
42	INH-OS-162	<i>Fallopia × bohemica</i>	site 1, Summerside, PEI, Canada	Oct 14, 2010	stem
43	INH-OS-163	<i>Fallopia japonica</i>	Dunstaffnage, PEI, Canada	Oct 14, 2010	stem
44	INH-OS-164	<i>Fallopia japonica</i>	Dunstaffnage, PEI, Canada	Oct 14, 2010	leaf
45	INH-OS-165	<i>Fallopia japonica</i>	Dunstaffnage, PEI, Canada	Oct 14, 2010	root
46	INH-OS-166	<i>Fallopia × bohemica</i>	site 1, Summerside, PEI, Canada	Oct 14, 2010	leaf
47	INH-OS-167	<i>Fallopia × bohemica</i>	site 1, Summerside, PEI, Canada	Oct 14, 2010	root
48	INH-OS-168	<i>Fallopia × bohemica</i>	St. Margaret's, PEI, Canada	Oct 14, 2010	stem
49	INH-OS-169	<i>Fallopia × bohemica</i>	St. Margaret's, PEI, Canada	Oct 14, 2010	leaf
50	INH-OS-170	<i>Fallopia × bohemica</i>	St. Margaret's, PEI, Canada	Oct 14, 2010	root
51	INH-368	<i>Fallopia japonica</i>	Dunstaffnage, PEI, Canada	Aug 30, 2011	root (old)
52	INH-369	<i>Fallopia japonica</i>	Dunstaffnage, PEI, Canada	Aug 30, 2011	root (young)
53	INH-367	<i>Fallopia japonica</i>	Dunstaffnage, PEI, Canada	Aug 30, 2011	stem
54	INH-366	<i>Fallopia japonica</i>	Dunstaffnage, PEI, Canada	Aug 30, 2011	leaf
55	INH-365	<i>Fallopia japonica</i>	Dunstaffnage, PEI, Canada	Aug 30, 2011	flower
56	INH-373	<i>Fallopia × bohemica</i>	St. Margaret's, PEI, Canada	Aug 30, 2011	root
57	INH-372	<i>Fallopia × bohemica</i>	St. Margaret's, PEI, Canada	Aug 30, 2011	stem
58	INH-371	<i>Fallopia × bohemica</i>	St. Margaret's, PEI, Canada	Aug 30, 2011	leaf
59	INH-370	<i>Fallopia × bohemica</i>	St. Margaret's, PEI, Canada	Aug 30, 2011	flower
60	INH-382	<i>Fallopia × bohemica</i>	site 2, Summerside, PEI, Canada	Aug 30, 2011	root (old)
61	INH-381	<i>Fallopia × bohemica</i>	site 2, Summerside, PEI, Canada	Aug 30, 2011	root (young)

Table 1. continued

sample	NRC-INH code	Latin name	origin/collection site	acquisition/collection time	plant part
62	INH-380	<i>Fallopia × bohemica</i>	site 2, Summerside, PEI, Canada	Aug 30, 2011	stem
63	INH-379	<i>Fallopia × bohemica</i>	site 2, Summerside, PEI, Canada	Aug 30, 2011	leaf
64	INH-378	<i>Fallopia × bohemica</i>	site 2, Summerside, PEI, Canada	Aug 30, 2011	flower
65	INH-374	<i>Fallopia × bohemica</i>	site 1, Summerside, PEI, Canada	Aug 30, 2011	root
66	INH-375	<i>Fallopia × bohemica</i>	site 1, Summerside, PEI, Canada	Aug 30, 2011	stem
67	INH-376	<i>Fallopia × bohemica</i>	site 1, Summerside, PEI, Canada	Aug 30, 2011	leaf
68	INH-377	<i>Fallopia × bohemica</i>	site 1, Summerside, PEI, Canada	Aug 30, 2011	flower
69	INH-OS-242	<i>Fallopia japonica</i>	Fraser Valley, BC, Canada	Aug 31, 2011	leaf
70	INH-OS-243	<i>Fallopia × bohemica</i>	Fraser Valley, BC, Canada	Aug 31, 2011	stem
71	INH-OS-238	<i>Fallopia × bohemica</i>	Fraser Valley, BC, Canada	Aug 31, 2011	leaf
72	INH-OS-241	<i>Fallopia japonica</i>	Fraser Valley, BC, Canada	Aug 31, 2011	root
73	INH-OS-248	<i>Polygonum polystachyum</i>	Fraser Valley, BC, Canada	Aug 31, 2011	root
74	INH-OS-247	<i>Polygonum polystachyum</i>	Fraser Valley, BC, Canada	Aug 31, 2011	stem
75	INH-OS-240	<i>Fallopia × bohemica</i>	Fraser Valley, BC, Canada	Aug 31, 2011	stem
76	INH-OS-239	<i>Fallopia × bohemica</i>	Fraser Valley, BC, Canada	Aug 31, 2011	root

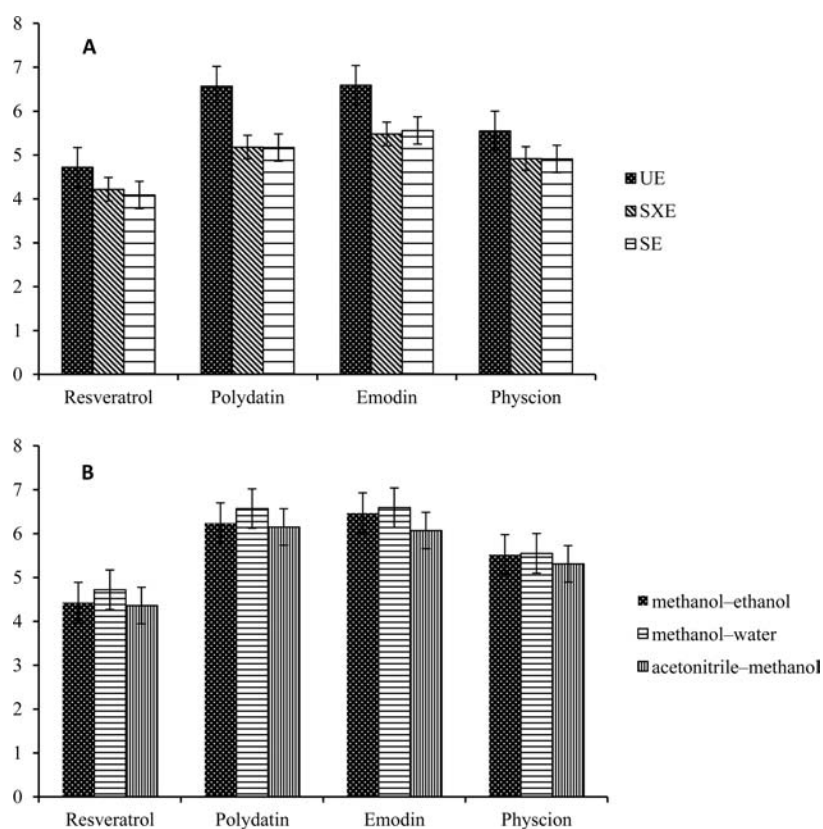


Figure 1. Effect of different extraction methods (A) and solvents (B) on contents of targeted compounds. UE, ultrasonic extraction; SEX, Soxhlet extraction; SE, soak extraction.

degasser, a quaternary pump, an autosampler, and a diode array detector (DAD), connected to a reversed-phase column (ZORBAX RRHD SB-C18 2.1 × 100 mm, 1.8 μm, Agilent USA). Data collection was performed using ChemStation (Agilent). An ultrasonic cleaner was used for sample extraction (VMR International, West Chester, PA, USA). The water used for all of the solutions and dilutions was prepared with a Millipore FONA84400 water purification system (USA). Methanol is of HPLC grade (Caledon Laboratories Ltd., Canada). Resveratrol, polydatin, emodin, and physcion (>98%) were purchased from Sigma-Aldrich (USA).

Knotweed Samples. Fourteen rhizome and root samples of *F. japonica* acquired commercially from 9 provinces in China, 54 samples of *F. japonica* and a related knotweed species from PEI, Canada, and 8 *F. japonica* and other knotweed samples (*F. × bohemica* and *F. polystachyum*)

from British Columbia (BC), Canada, were investigated (Table 1). Voucher specimens for knotweed samples collected in PEI have been kept at the National Research Council Canada.

Following closer taxonomical investigations, we were able to reveal two distinct taxa among the PEI samples, *Fallopia japonica* (Houtt.) Ronse Decraene and the hybrid *F. × bohemica* (Chrtek & Chrtková) J. P. Bailey. On the basis of previous systematic studies,^{24–26} we identified some samples and came across numerous populations of *F. × bohemica*. Description of the voucher specimens can be helpful for researchers involved in natural health products and members of industries who might want to address harvesting, cultivation, and processing of specific taxa/samples.

F. japonica (Houtt.) Ronse Decraene var. *japonica* (NRC voucher specimen code INH-365-369) is a rhizomatous shrub bearing rather

Table 2. Orthogonal Design and Statistical Analysis for Optimization of Extraction Conditions

(A) Factors and Levels of Orthogonal Design				
factor	solvent/sample ratio (mL/g) (A)	extraction time (min) (B)	methanol/water ratio (v/v) (C)	times of extraction (D)
1	25:1	10	50:50	1
2	30:1	20	60:40	2
3	35:1	30	70:30	3

(B) Arrangement and Results of L9 (3 ⁴) Orthogonal Test									
no.	factors				content (mg/g)				
	A	B	C	D	resveratrol	polydatin	emodin	physcion	total
1	25:1	10	50:50	1	5.66	4.28	5.63	5.12	20.69
2	25:1	20	60:40	2	5.11	4.05	5.51	3.82	18.49
3	25:1	30	70:30	3	4.88	4.36	5.06	4.18	18.48
4	30:1	10	60:40	3	4.29	2.99	3.81	4.02	15.11
5	30:1	20	70:30	1	4.52	3.84	4.79	3.43	16.58
6	30:1	30	50:50	2	4.12	3.8	5.19	4.15	17.26
7	35:1	10	70:30	2	3.7	3.33	4.55	3.73	15.31
8	35:1	20	50:50	3	3.38	3.52	4.33	4.87	16.10
9	35:1	30	60:40	3	3.57	4.06	4.01	4.26	15.90
K ₁	57.66	51.11	54.05	53.17					
K ₂	48.95	51.17	49.50	51.06					
K ₃	47.31	51.64	50.37	49.69					
R	10.35	0.53	4.55	3.48					

(C) Analysis of Variance (ANOVA) Results				
source	sum of squares	degrees of freedom	F value	P value
A ^a	20.63	2	367.38	<0.05
B	0.056	2	1.00	>0.05
C ^a	3.88	2	69.26	<0.05
D ^a	2.04	2	36.48	<0.05
total	26.61	8		

^aSignificant ($P < 0.05$), $F_{0.05}(3, 2) = 19.16419$.

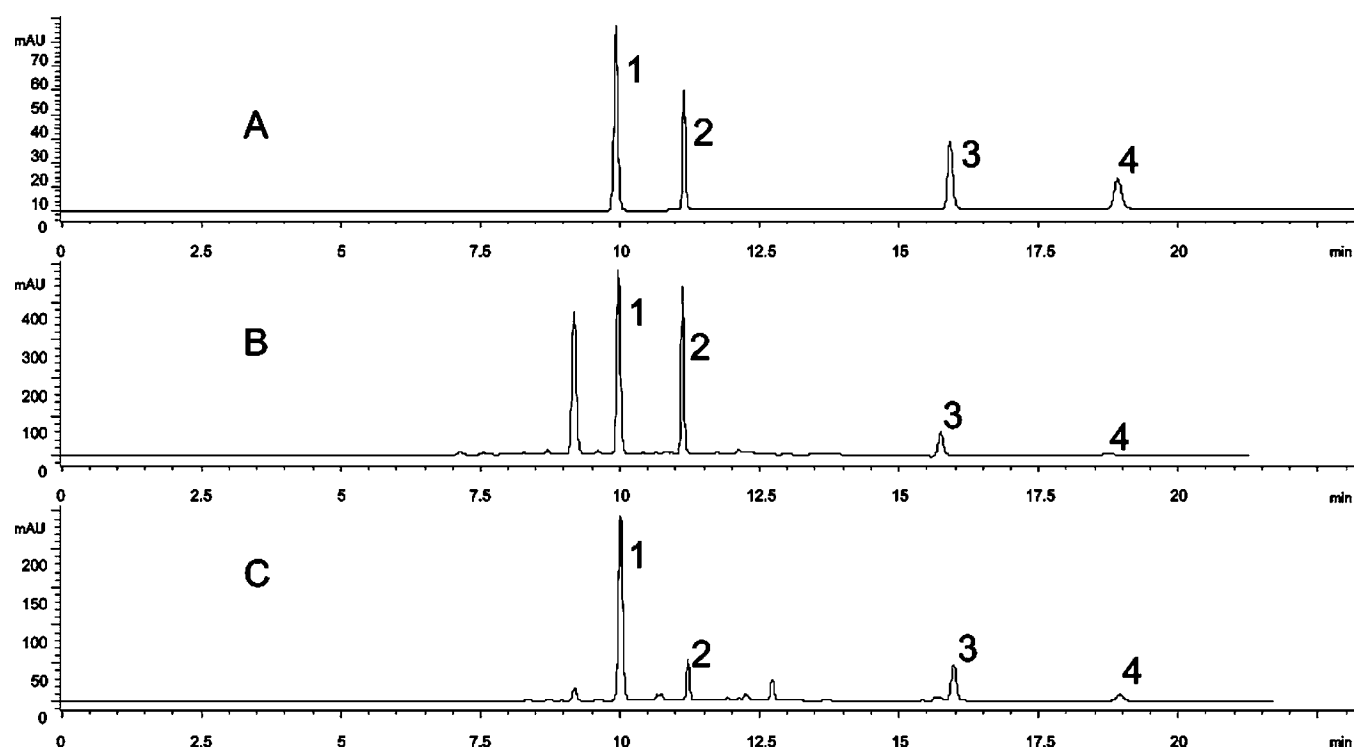


Figure 2. UPLC chromatograms of standard compounds (A, peak 1, polydatin; 2, resveratrol; 3, emodin; and 4, physcion), PEI-grown Japanese knotweed extract (B), and extract of *F. japonica* from China (C).

Table 3. Contents of Resveratrol, Polydatin, Emodin, and Physcion from Different Samples ($n = 3$)

sample	polydatin (mg/g)	resveratrol (mg/g)	emodin (mg/g)	physcion (mg/g)	sample	polydatin (mg/g)	resveratrol (mg/g)	emodin (mg/g)	physcion (mg/g)
1	6.57 ± 0.12	4.72 ± 0.09	6.59 ± 0.08	5.55 ± 0.08	39	0.10 ± 0.01	0	0	0
2	6.53 ± 0.11	2.77 ± 0.07	4.48 ± 0.06	2.59 ± 0.05	40	0.13 ± 0.01	0	0	0
3	12.55 ± 0.19	2.92 ± 0.06	7.23 ± 0.10	9.13 ± 0.11	41	13.47 ± 0.30	2.01 ± 0.06	1.41 ± 0.03	2.30 ± 0.08
4	12.33 ± 0.20	2.89 ± 0.07	9.02 ± 0.6	15.27 ± 0.23	42	0.07 ± 0.01	0	0.02 ± 0.01	0
5	8.87 ± 0.15	3.41 ± 0.07	5.94 ± 0.10	12.66 ± 0.21	43	0.10 ± 0.02	0	0	0
6	5.72 ± 0.11	2.88 ± 0.09	4.37 ± 0.07	6.29 ± 0.18	44	0.25 ± 0.04	0	0.03 ± 0.01	0
7	8.77 ± 0.13	6.39 ± 0.07	4.08 ± 0.06	3.73 ± 0.08	45	8.28 ± 0.18	6.27 ± 0.13	6.85 ± 0.06	10.89 ± 0.16
8	10.45 ± 0.16	3.94 ± 0.06	6.15 ± 0.09	10.23 ± 0.10	46	0.41 ± 0.03	0	0	0
9	8.82 ± 0.11	5.50 ± 0.07	7.79 ± 0.08	12.37 ± 0.18	47	9.34 ± 0.17	2.74 ± 0.07	0.27 ± 0.02	1.15 ± 0.06
10	9.20 ± 0.14	2.06 ± 0.03	6.45 ± 0.09	15.72 ± 0.28	48	0.03 ± 0.01	0	0	0
11	8.57 ± 0.12	2.76 ± 0.04	6.05 ± 0.07	12.86 ± 0.21	49	0.06 ± 0.01	0.01 ± 0.01	0	0
12	12.01 ± 0.07	12.07 ± 0.13	8.39 ± 0.06	12.84 ± 0.20	50	0.43 ± 0.03	0.05 ± 0.01	0	0
13	7.61 ± 0.11	3.71 ± 0.05	8.25 ± 0.07	11.30 ± 0.17	51	12.18 ± 0.22	4.31 ± 0.03	2.74 ± 0.05	8.98 ± 0.29
14	11.73 ± 0.19	4.11 ± 0.04	9.23 ± 0.10	13.84 ± 0.21	52	3.11 ± 0.02	1.36 ± 0.04	0.55 ± 0.02	3.97 ± 0.06
15	0.74 ± 0.02	0.11 ± 0.02	0.54 ± 0.05	1.52 ± 0.09	53	0.11 ± 0.01	0	0.06 ± 0.01	0.08 ± 0.01
16	11.40 ± 0.25	0.83 ± 0.05	3.59 ± 0.11	15.54 ± 0.30	54	0.15 ± 0.02	0	0	0.33 ± 0.01
17	0.30 ± 0.05	0.15 ± 0.02	0.41 ± 0.03	0.77 ± 0.03	55	0	0	0	0
18	0.56 ± 0.07	0.14 ± 0.02	0.34 ± 0.03	1.04 ± 0.08	56	12.95 ± 0.19	2.17 ± 0.03	1.48 ± 0.06	2.33 ± 0.03
19	10.34 ± 0.21	0.85 ± 0.04	4.58 ± 0.15	15.42 ± 0.28	57	0.08 ± 0.01	0	0.05 ± 0.01	0
20	0.16 ± 0.03	0.03 ± 0.01	0.06 ± 0.01	0.16 ± 0.01	58	0.05 ± 0.01	0	0	0
21	0.28 ± 0.05	0.03 ± 0.01	0.11 ± 0.01	0.39 ± 0.04	59	0	0	0	0
22	7.18 ± 0.20	0.56 ± 0.02	4.60 ± 0.09	9.71 ± 0.20	60	13.26 ± 0.21	2.35 ± 0.03	1.79 ± 0.03	2.48 ± 0.03
23	0.20 ± 0.02	0.05 ± 0.01	0.12 ± 0.01	0.24 ± 0.02	61	13.68 ± 0.27	2.28 ± 0.07	1.32 ± 0.03	2.69 ± 0.05
24	0.04 ± 0.04	0	0.03 ± 0.01	0.07 ± 0.01	62	0.09 ± 0.01	0	0.05 ± 0.01	0
25	0.13 ± 0.02	0	0.05 ± 0.01	0.13 ± 0.02	63	0.17 ± 0.01	0	0	0
26	0.06 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	64	0	0	0	0
27	12.16 ± 0.27	1.06 ± 0.09	6.51 ± 0.13	14.54 ± 0.21	65	1.13 ± 0.03	0.18 ± 0.01	0.54 ± 0.02	1.25 ± 0.05
28	0.67 ± 0.05	0.07 ± 0.04	0.54 ± 0.06	1.43 ± 0.10	66	0.11 ± 0.01	0	0.08 ± 0.01	0
29	0.08 ± 0.01	0	0.04 ± 0.01	0.04 ± 0.01	67	0.06 ± 0.01	0	0	0
30	0.09 ± 0.01	0.01 ± 0.01	0.04 ± 0.01	0	68	0	0	0	0
31	11.88 ± 0.25	1.12 ± 0.05	5.42 ± 0.11	7.20 ± 0.14	69	0.07 ± 0.01	0	0	0
32	0.13 ± 0.01	0	0.05 ± 0.01	0.06 ± 0.01	70	0.07 ± 0.01	0	0.09 ± 0.01	0
33	0.01 ± 0.01	0	0	0	71	0.06 ± 0.01	0	0	0
34	0.13 ± 0.02	0	0.01 ± 0.01	0	72	6.33 ± 0.13	1.28 ± 0.02	0.67 ± 0.02	0.82 ± 0.03
35	0.62 ± 0.01	0.08 ± 0.01	0.64 ± 0.07	1.17 ± 0.08	73	8.25 ± 0.14	1.97 ± 0.05	1.21 ± 0.05	0.67 ± 0.02
36	0.08 ± 0.01	0	0.04 ± 0.01	0.08 ± 0.01	74	0.09 ± 0.01	0	0	0.03 ± 0.01
37	0.16 ± 0.02	0	0.03 ± 0.01	0.89 ± 0.03	75	0.07 ± 0.01	0	0.05 ± 0.01	0
38	13.38 ± 0.24	2.13 ± 0.05	2.65 ± 0.10	9.49 ± 0.19	76	6.72 ± 0.12	1.39 ± 0.03	0.78 ± 0.03	0.85 ± 0.03

small, acuminate, entire leaves with blades showing truncate bases. Limbs are slightly longer than wide, no longer than 11 cm on the voucher specimen. Veins on the abaxial surface are devoid of hairs or trichomes; they show irregular knobs as shown in Zika and Jacobson.²⁶ The presence or absence of hairs or knobs is the best character that differentiates *Fallopia sachalinensis* and *F. × bohemica* from *F. japonica* var. *japonica*. The inflorescences are loose and longer than the subtending leaf.

All of the other specimens were identified as *Fallopia × bohemica* (Chrtek & Chrteková) J. P. Bailey (NRC voucher specimen codes INH-370-373, INH-374-377, INH-378-382), although they showed differences in relation to the size of blades and the degree of compactness of inflorescences. The specimens bearing the accession number INH-370-373 as well as INH-374-377 possess blades of 15 cm or longer with truncate bases (or very slightly cordate); the apex is acuminate. Inflorescences are compact and short (much shorter than the subtending leaf). On the abaxial surface, short hairs are conspicuous along the veins, which is characteristic of the hybrid. The last specimen (INH-378-382) shows characters from the species and the hybrid. Leaves are shorter than the latter specimens and oscillate around 12 cm long. Bases are again truncate (or slightly cordate), and blades terminate with an acuminate apex. The presence of short hairs instead of knobs and the fact that the inflorescences are slightly shorter than the subtending

leaf indicate that we have the hybrid. Although shorter, inflorescences are less compact than the other *F. × bohemica* specimens.

Sample Preparation. Fresh samples were cleaned with water and then milled into powder by a versatile plant grinder after being air-dried and then oven-dried (50–60 °C) right before grinding. Before weighing out, all samples were further dried on a freeze-dryer to remove moisture.

Extraction Method. A 0.4 g aliquot of dry sample was accurately weighed out, and 10 mL of methanol/water (50:50, v/v) was added; the sample was then placed into an ultrasonic cleaner. The mixture was extracted for 30 min, and then the extraction solution was centrifugally separated and filtered through 0.45 μm Millipore nylon membrane, and an aliquot of the filtrate was used for UPLC analysis.

Preparation of Reference Substance Solutions. Resveratrol, polydatin, emodin, and physcion are the common components reportedly present in *F. japonica*, so they were commercially sourced and used as the reference substances in this study. Stock solutions of resveratrol (0.20 mg/mL), polydatin (0.60 mg/mL), emodin (0.25 mg/mL), and physcion (0.10 mg/mL) were prepared by dissolving the pure compounds in methanol. After filtering through a 0.45 μm Millipore membrane, the reference substance solution was directly used for UPLC analysis.

UPLC Analysis. A reverse phase SB-C18 column (2.1 × 100 mm, 1.8 μm) from Agilent was used with the mobile phase consisting of

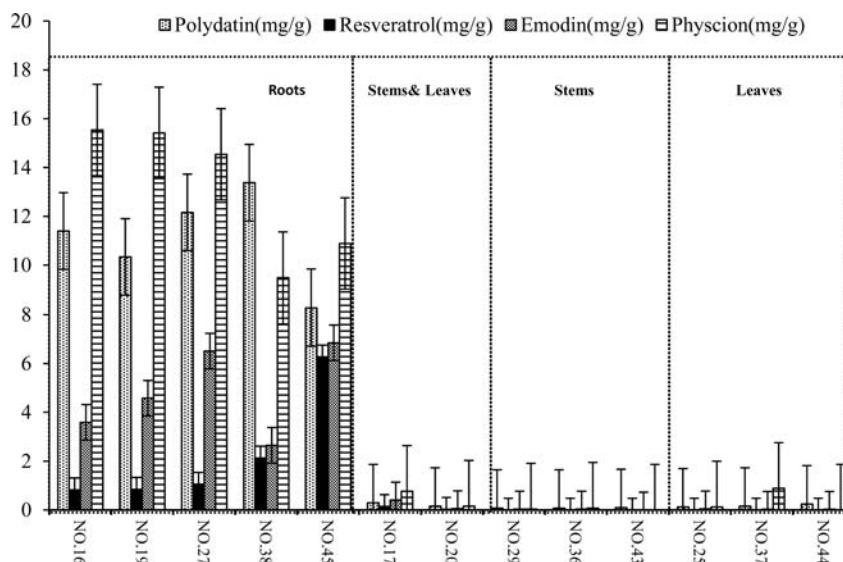


Figure 3. Contents of four targeted compounds in different *F. japonica* samples collected from Dunstaffnage, PEI, Canada.

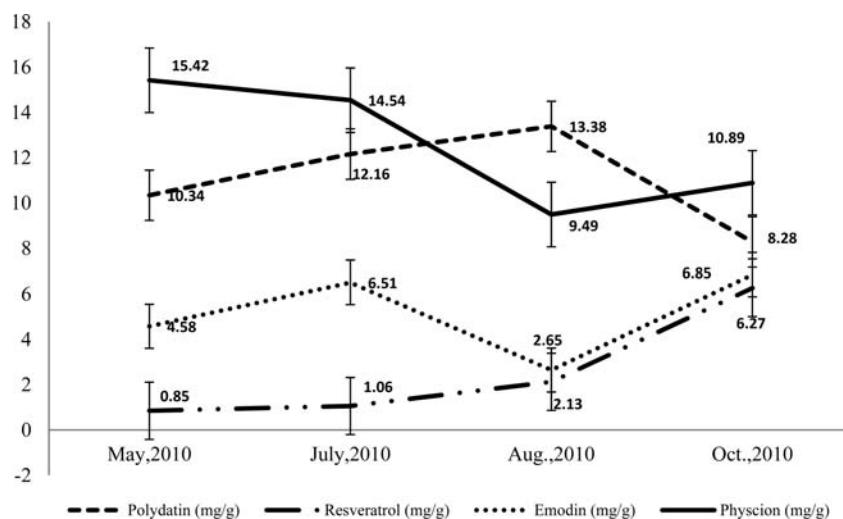


Figure 4. Levels of four targeted compounds in roots of *F. japonica* collected at different times in PEI, Canada.

methanol (A) and water (B). The optimized gradient elution was performed using the following linear gradient: 0 min, 15% A; 3 min, 20% A; 5 min, 25% A; 8 min, 40% A; 11 min, 80% A; 15 min, 90% A; 17 min, 90% A. The column compartment was kept at a temperature of 50 °C, detection wavelength was set at 303 nm, and the volume of sample injected was 2 μ L.

Multivariate Data Analysis. The data set from UPLC analysis was imported to SIMCA-P+ (version 12.0, Umetrics, Umea, Sweden) for conducting partial least-squares discriminant analysis (PLS-DA). Quantified compound concentrations were all mean-centered and Pareto-scaled prior to the analysis.

RESULTS AND DISCUSSION

Optimization of UPLC-DAD Method. To obtain optimal conditions for separation and quantification, the mobile phase and its flow rate, conditions for elution, column temperature, and detection wavelength were investigated in this study.

The chromatographic conditions were optimized on the basis of conditions given in the literature.^{27,28} In the gradient optimization, gradient time, shape, and initial composition of the mobile phase were taken into consideration, and the optimized gradient elution was presented above under UPLC

Table 4. Average Contents of Four Targeted Compounds in *F. japonica* Root Samples from China and PEI, Canada

compound	Chinese samples (mg/g)	PEI samples (mg/g)	PEI samples (Oct) (mg/g)
polydatin	9.27 \pm 1.02	11.04 \pm 1.35	10.69 \pm 1.17
resveratrol	4.3 \pm 0.16	2.68 \pm 0.18 ^a	4.25 \pm 0.14
emodin	6.72 \pm 0.25	5.15 \pm 0.21 ^a	5.13 \pm 0.22 ^a
physcion	10.31 \pm 0.68	12.58 \pm 0.71 ^a	12.67 \pm 0.58 ^a

^a*, significant ($P < 0.05$).

Analysis. Column temperatures of 45, 50, and 55 °C and flow rates of 0.4, 0.5, 0.8, and 1.0 mL/min were also investigated. Considering system back pressure and running time, the flow rate was set at 0.5 mL/min when the column temperature was kept at 50 °C. Also, the detection wavelength was examined. When the wavelength was at 303 nm, most components showed strong responses; thus, the DAD detection was performed at 303 nm.

Under these optimal conditions, resveratrol, polydatin, emodin, physcion, and other minor components in the extracts of *F. japonica* samples were well separated. All method validation

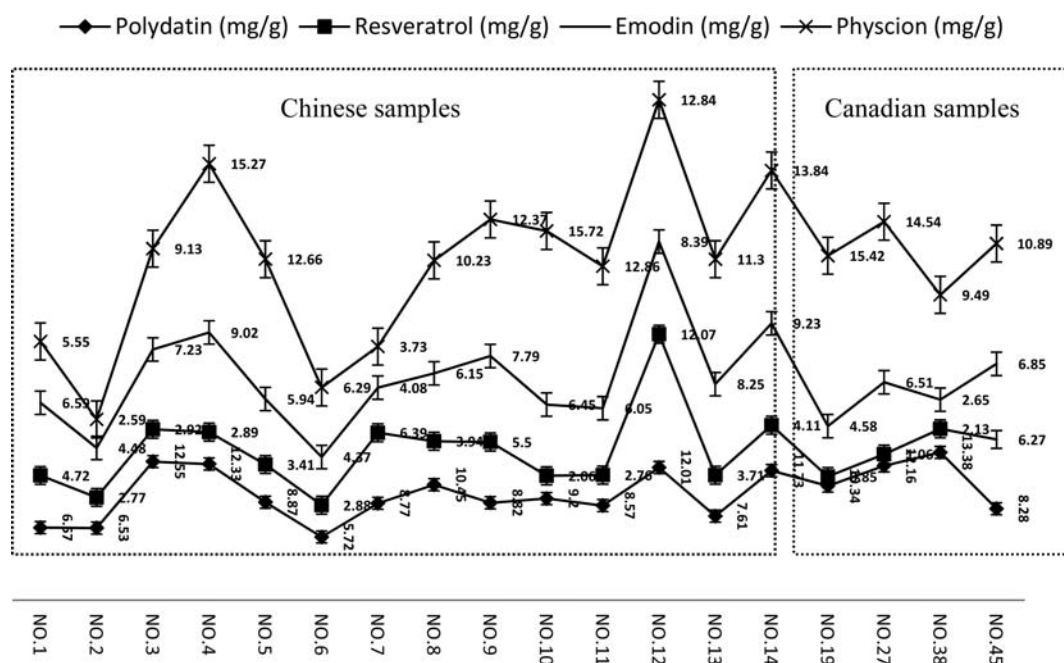


Figure 5. Contents of four targeted compounds in *F. japonica* root samples from China and PEI, Canada.

tests below were carried out on *F. japonica* extracts prepared as described above. The injection precision was determined by repeating UPLC injection of the same sample solution six times per day. The sample stability was determined with measurements from a single sample solution stored at room temperature for 2, 4, 6, 8, and 12 h. The repeatability was assessed by analyzing six separate samples.

The relative peak area (RPA) of each characteristic peak was calculated for the estimation of injection precision, stability, and repeatability and the results were as follows: for injection precision, the relative standard deviations (RSD) of the RPA < 0.56%; for sample stability, RSD < 1.68%; for repeatability, RSD < 1.71% for six independent samples. Thus, the results indicated that the UPLC-DAD method was suitable for this quantitative analysis work.

Optimization of Extraction Conditions. To exhaustively extract resveratrol, polydatin, emodin, and physcion from *F. japonica*, three extraction approaches were compared: ultrasonic extraction (UE), conventional Soxhlet extraction (SXE), and soak extraction (SE). The extraction efficiencies of resveratrol, polydatin, emodin, and physcion using the three proposed extraction methods were measured, as milligrams per gram of raw sample, to be 4.72, 6.57, 6.59, and 5.55 mg/g; 4.22, 5.18, 5.48, 4.92 mg/g; and 4.09, 5.17, 5.56, and 4.91 mg/g, respectively (Figure 1A). Obviously, ultrasonic extraction had the highest extraction efficiency, which was then chosen as the extraction approach for the following experiments.

Then, the extraction solvent, a key factor for UE, was evaluated. Three solvent mixtures were tested, methanol–ethanol, methanol–water, and acetonitrile–methanol, in a ratio of 80:20 (v/v) to evaluate their efficiency for extraction of resveratrol, polydatin, emodin, and physcion from *F. japonica*.

After ultrasonic extraction, the levels of resveratrol, polydatin, emodin, and physcion by using the three solvent mixtures were found to be 4.43, 6.24, 6.47, and 5.52 mg/g; 4.72, 6.57, 6.59, and 5.55 mg/g; and 4.36, 6.15, 6.07, and 5.31 mg/g, respectively (Figure 1B). This showed that the extraction of resveratrol, polydatin, emodin, and physcion with methanol–water (80:20)

was the best. For dry plant materials, solvents such as methanol–water with higher polarity could penetrate plant cell membranes and enhance the extraction of bioactive components. Therefore, the methanol–water (80:20) was chosen as the solvent for further investigation.

Subsequently, the effects of different factors on ultrasonic extraction of resveratrol, polydatin, emodin, and physcion were investigated, and orthogonal experiment design was used to determine the significance of four environmental factors including solvent/sample ratio (mL/g), methanol/water ratio (v/v), extraction time (min), and times of extraction (number of times).

The experimental factors with corresponding levels are presented in Table 2A, and the results of orthogonal design L_9 (3^4) are presented in Table 2B. The intuitive analysis from R value in Table 2B indicates that the influence order of each variable on the extraction efficiency is $A > C > D > B$. It also showed that K_1 had the highest K value in factor A, which means that compared with other levels of factor A, level 1 has the highest extraction efficiency and is the most suitable level for extraction. On the basis of the same principle, level 3 of factor B, level 1 of factor C, and level 1 of factor D were chosen for extraction. To verify the data analysis of orthogonal experiment design, analysis of variance (ANOVA) was used to further determine which factor was significant in affecting the extraction yield of resveratrol, polydatin, emodin, and physcion. As shown in Table 2C, factors A, C, and D are clearly the most significant ones ($P < 0.05$) affecting extraction.

The orthogonal experiment design combined with statistical analysis had been applied to optimize the conditions of extraction for improving the yield for resveratrol, polydatin, emodin, and physcion in *F. japonica*, and the optimal extraction condition was used as described above.

Results of Analysis. *Chromatograms of Reference Compounds and F. japonica Extract.* *F. japonica* contains emodin, emodin-6-ether, emodin-8-monomethyl ether, chrysophanol, rhein, emodin-8- β -D-glucoside, physcion, resveratrol, and polydatin, with resveratrol, polydatin, emodin, and physcion

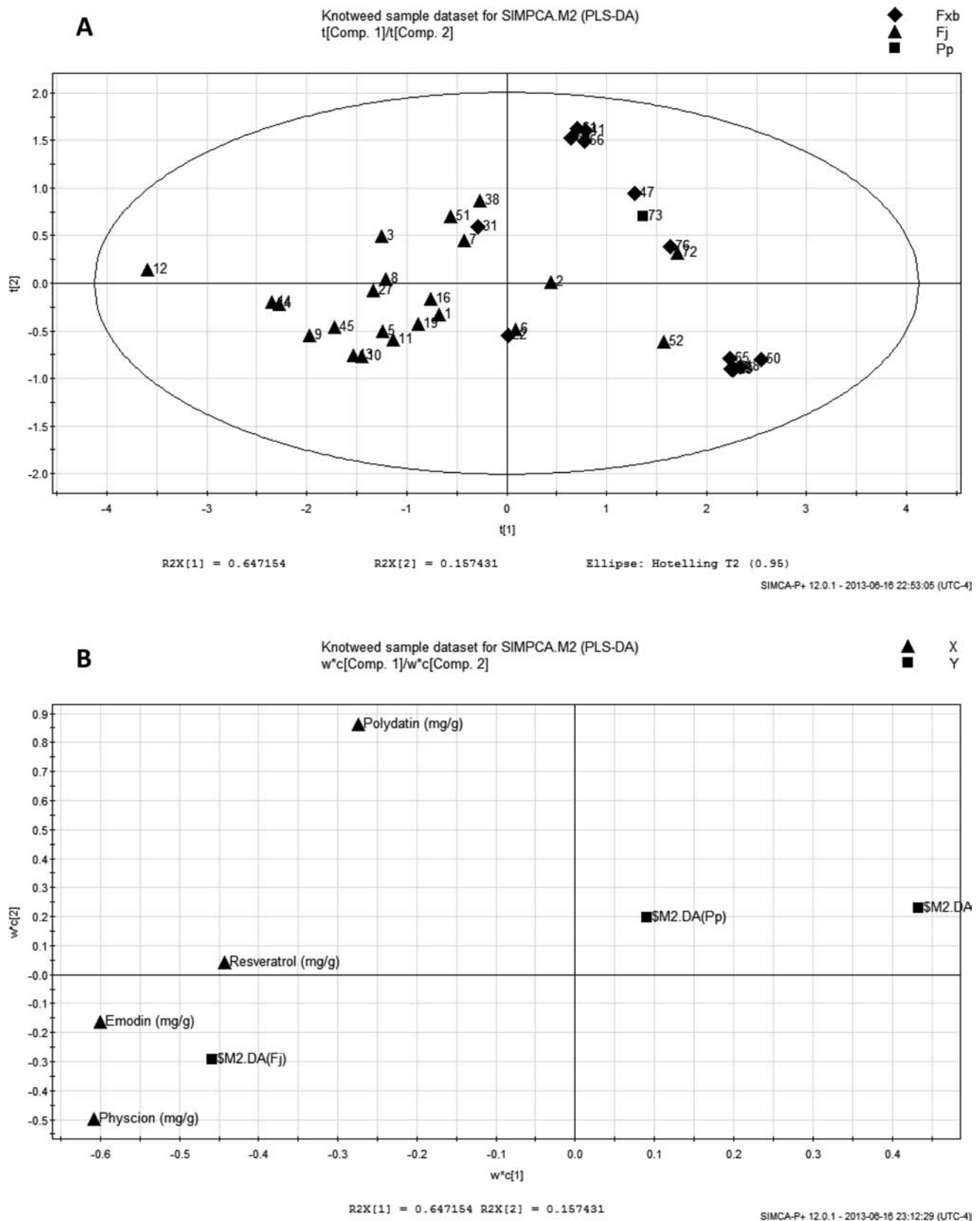


Figure 6. PLS-DA score plot (A) and loading plot (B) showing clusters based on contents of polydatin, resveratrol, emodin, and physcion in root samples of *F. japonica* (Fj), *F. × bohemica* (Fxb), and *Polygonum polystachyum* (Pp) from China and Canada.

as the main and biologically active components. To identify the main components of knotweed species grown in PEI, the UPLC profiles of the standard compounds resveratrol, polydatin,

emodin, and physcion (Figure 2A) are compared with the extracts of PEI-grown *F. japonica* (Figure 2B) and *F. japonica* from China (Figure 2C). On the basis of UPLC analysis, PEI-

grown knotweed shows a similar composition profile in comparison with the Chinese samples except that it contains an additional, more polar analogue of resveratrol, identified as resveratrolside²⁹ (based on NMR and MS analysis of pure compounds obtained through preparative HPLC, data not shown).

Contents of Resveratrol, Polydatin, Emodin and Physcion in Different Plant Parts of Knotweed Grown in PEI, Canada. It is well-known that different parts of a raw herb may have somewhat different composition profiles and thus may be used for different therapeutic purposes. In this study, the contents of resveratrol, polydatin, emodin, and physcion of various parts of *F. japonica* grown in PEI were measured and compared. Root, stem, flower, and leaf samples of *F. japonica* collected from PEI were extracted and analyzed on UPLC. The contents of resveratrol, polydatin, emodin, and physcion are shown in Table 3. The contents of resveratrol, polydatin, emodin, and physcion were found to vary considerably in various parts of the plant and samples collected during different seasons (Figure 3).

Contents of Resveratrol, Polydatin, Emodin, and Physcion in the Root of *F. japonica* Harvested at Different Times in PEI, Canada. As shown above, different plant parts contain various levels of resveratrol, polydatin, and emodin, and the root was found to contain higher levels of these compounds. Therefore, we studied the effect of harvesting time on the levels of resveratrol, polydatin, emodin, and physcion various in root samples of *F. japonica*, and the results are shown in Figure 4. The results indicated that polydatin in *F. japonica* increases from May to July, reaches its highest in August, and then begins to decrease. Resveratrol, on the other hand, gradually increased from May to October, and the level was 2.5 times higher in October than in May. However, emodin in *F. japonica* appeared not to have a particular trend of accumulation during the year, whereas physcion seemed to decrease gradually from May to October.

Comparison on Contents of Polydatin, Resveratrol, Emodin, and Physcion among Root Samples of *F. japonica* from China and PEI, Canada. It is well-known that different growth environments have an enormous impact on the content of active ingredients in plants.³⁰ China is in eastern Asia and Canada is located in North America, so there is a great difference of climate and environmental factors related to plant growth. It is thus very interesting to compare the levels of these key components among the *F. japonica* root samples.

As shown in Table 4 and Figure 5, polydatin level was found to be within a similar range in both Chinese and PEI samples; the average content of polydatin in the root of *F. japonica* grown in PEI is about 11.04 mg/g, slightly higher than the average of Chinese samples (9.27 mg/g). The level of resveratrol in the Chinese and PEI samples differs considerably, with the average content in Chinese samples being 4.30 mg/g, 1.6 times higher than that of the PEI samples. However, if we choose only the samples harvested in October for comparison, the contents of resveratrol appeared to be at similar levels. Chinese samples also contain a higher level (1.3 times) of emodin and less physcion than the PEI samples (6.72 mg/g emodin and 10.31 mg/g physcion in Chinese samples; and 5.15 and 12.58 mg/g in PEI samples).

Comparison of Contents of Polydatin, Resveratrol, Emodin, and Physcion among Root Samples of *F. japonica*, *F. × bohemica*, and *Polygonum polystachyum* Collected at Different Sites from PEI, and Acquired from BC, Canada, and China. In this study, we compared the contents of polydatin, resveratrol, emodin, and physcion in all root samples, including

20 samples collected at three different locations (4 sites) in PEI, Canada, 3 samples acquired from BC, Canada (as shown in Table 1), and 14 samples from China. The results indicated that the contents of resveratrol and polydatin were higher in Japanese knotweed, *F. japonica*, grown in PEI or from China. In comparison, we observed much lower levels of these compounds in other related species, *F. × bohemica* and *P. polystachyum*, with a difference of >10 times in some samples.

This difference of key metabolite abundances was further demonstrated by using a multivariate data analysis tool, which showed a fairly clear separation of *F. japonica* from other related species, based on levels of the four compounds analyzed in root samples. As shown in Figure 6, the score and loading plots (A and B) of the partial least-squares discriminant analysis (PLS-DA) demonstrated that *F. japonica* contains distinctly higher levels of resveratrol, polydatin, emodin, and physcion. One sample (no. 72), acquired from BC, Canada, as *F. japonica*, seems to contain lower levels of resveratrol and other compounds, indicating the effect of environmental factors.

In summary, this study showed that the content of resveratrol in Japanese knotweed (*F. japonica*) grown in PEI, Canada, varies in different parts of the plant and that the root contains a higher level of resveratrol than stem and leaf. The level of resveratrol in root samples of *F. japonica* from PEI is comparable to that of Chinese samples, whereas a significant difference was found between the common Japanese knotweed (*F. japonica*) and its closely related species *F. × bohemica*. Japanese knotweed contains >10 times the level of resveratrol as *F. × bohemica*. Also, time of collection was found to have a great influence on the contents of resveratrol; resveratrol level is at its highest in October. Additionally, PEI-grown knotweed contains a similar levels of resveratrol and polydatin as the Chinese samples collected in the month of October, but the contents of emodin and physcion are statistically different (lower for emodin and higher for physcion). As such, we conclude that Japanese knotweed grown in PEI, Canada, is a commercially viable source of raw material for resveratrol production.

■ AUTHOR INFORMATION

Corresponding Author

*(J.Z.) Present address: Aquatic and Crop Resource Development, National Research Council Canada, 1411 Oxford Street, Halifax, NS B3H 3Z1, Canada. Phone: +1-902-426-7408. Fax: +1-902-426-9413. E-mail: junzeng.zhang@nrc.gc.ca.

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Notes

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