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The Study of Phenolic Compounds and Antioxidant Activity of Raw Materials of *Reynoutria Sachalinensis* (F. Schmidt) Nakai

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ABSTRACT

Phenolic composition and quantitative evaluation of herbal part and root extracts for *Reynoutria sachalinensis* studied by HPLC method. Using the same method, we also established the antioxidant activity of *R. sachalinensis* raw materials. Six phenolic compounds were idenified for herbal part of *R. sachalinensis* as, gallic acid, chlorogenic acid, *trans*-cinnamic acid, rutin, hyperoside and isoquercitrin at total amount of 885.37±21.25 mg/kg. Neochlorogenic acid and rutin were found as main compounds for herbal part of *R. sachalinensis,* and gallic acid and 6,7-dihydroisoflavone were determined for root of *R. sachalinensis*. In *R. sachalinensis* roots we found gallic acid and 6,7-dihydroisoflavone.

The HPLC study of antioxidant activity showed almost identical antioxidant potential of bioactive substances (BASs) in *R. sachalinensis* herbal parts and roots that is 3.85±0.09 and 3.59±0.09 mg/g in Trolox equivalent respectively.

The obtained data proved the feasibility of new antioxidant drugs development on the basis of *R. sachalinensis* raw materials.

Keywords: *Reynoutria sachalinensis*, phenolic compounds, HPLC

INTRODUCTION

Reynoutria sachalinensis (F. Schmidt) Nakai (synonyms: *Polygonum sachalinensis* F. Schmidt, *Fallopia sachalinensis* (F. Schmidt) Ronse Decr., *Pleuropterus sachalinensis* (F. Schmidt) H. Gross, *Tiniaria sachalinensis* (F. Schmidt)

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Janch.) is a perennial herbaceous plant of *Polygonaceae* family. The plant originates from East Asia, growing in Korea, Japan, the Kurile Islands and Sakhalin. This species first appeared in Europe in 1855 and has been grown at botanical gardens as an ornamental plant. *R. sachalinensis* is met as a weed in many European countries, including Ukraine[1, 2].

Anthraquinones (physcion, I-O-methylemodin, emodin) and stilbene (*trans*-resveratrol) possessing cytotoxic activity were separated from methanol extract of herbal part of *R. sachalinensis*[3].

Another species of *Reynoutria* genus which is also under study, *R. japonica,* contains phenolic compounds, including flavonoids, anthraquinones, condensed tanning agents and stilbenes, polysaccharides. Extracts from *R. japonica* demonstrate antipyretic, analgesic and anti-inflammatory activities[4].

HPLC/UV/ESI-MS studies of the rhizomes of *R. japonica*, *R. sachalinensis and Reynoutria* x *bohemica* revealed 171 compounds, comprising stilbenes, carbohydrates, procyanidins, flavan-3-ols, anthraquinones, phenylpropanoids, lignin oligomers, hydroxycinnamic acids, naphthalenes and their derivatives[5].

Antimicrobial activity of acetone extract from rhizome of *R. japonica* and *R. sachalinensis*, as well as of their hybrid *Reynoutria* x *bohemica* was studied as regards the caries-inducing pathogens, against *Streptococcus mutans*. The most active extract was found for *R. japonica rhizome*[6].

In Ukraine R. sachalinensis is not a pharmacopoeia-registered plant, still, issuing from the experience of folk medicine application of this plant in East Asian countries as well as from the results of scientific research, we may foresee its feasibility for medical drugs development.

As the oxidative stress in human organism may provoke the diseases of different severity, including cancers, atherosclerosis, neurodegenerative diseases (Parkinson's, Alzheimer's, etc.), hypertension, diabetes mellitus, cardiovascular diseases, reproductive system dysfunctions, etc, search of promising antioxidants of herbal origin has become an important aspect in pharmacy[7-12].

Antioxidant activity were studied of phenolic compounds as, anthraquinones (emodin, emodin-8-O-beta-D-glucopyranoside and physcion-8-O-beta-D-glucopyranoside) and flavonoids (quercetin-3-O-alpha-L-arabinofuranoside, quercetin-3-O-beta-D-galactopyranoside and quercetin-3-O-beta-D-glucuronopyranoside) for flower extracts of *R. sachalinensis in* our previous study[13].

Therefore, for deeper understanding of *R. sachalinensis* application prospects, it turned out feasible to study phenolic substances in herbal and roots extracts

of this plant as well as its antioxidant activity. We conducted a comparative study of *R. sachalinensis* herbal parts and roots.

METHODOLOGY

Plant materials

In experiments we used air-dried milled roots and herbal parts of *R. sachalinensis*. Herbal parts were collected within blossoming period in June, roots – in September in Kharkiv Region, Ukraine during 2018-2019.

The plant material sample was identified by Prof. *Tatyana* Gontova, Department of Botany, National University of Pharmacy, Ukraine and voucher specimens were deposited at National University of Pharmacy, Ukraine.

Extraction

Extracts for analysis were prepared by extracting 0.3 g milled raw material with 10 mL methanol within 20 min on ultrasonic bath at 20±2°C. The obtained extracts were filtered through a membrane filter (0.45 μm)[14].

General experimental procedures

For study of phenolic compounds in *R. sachalinensis*, as well as determination of antioxidant activity by HPLC method (Waters Corporation, Milford, USA) with Waters 996 PDA photodiode matrix detector, (Waters Corporation, USA), Wise Clean WUC-A06H ultrasonic cleaning set (**Daihan**, Korea), ANG 100 analytical balance (AXIC, Poland), standard samples of substances and solvents for chromatographic analysis from Merck KGaA (Darmstadt, Germany).

Chromatographic analysis by HPLC

Chromatographic separation of phenolic compounds was performed using ACE 5 C18 column 250 mm × 4.6 mm (Pennsylvania, USA). Elution flow rate was 1 ml/min. Mobile phase binary solvent system consisted of solvent A (0.1% acetic acid aqueous solution) and solvent B (acetonitrile). All solvents passed ultrasonic degassing and 0.23 μm pore size membrane filter. Linear gradient program looked as follows:

Time, min	Solvent A, %	Solvent B, %
0–8	5–15	95-85
8–30	15–20	85-80
30–48	20–40	80-60
48–58	40–50	60-50
58–65	50	50
65–66	50–95	50-5

The column had constant temperature of 25°C. 10 μl samples were injected[13].

For determination of antioxidant activity after application of HPLC-PDA detector system the mobile phase containing tested samples was fed with Gilson 305 pump (Middleton, WI, USA) to the column via mixing tee with ABTS reagent in split relation 1:1. A Teflon column (Waters PCR module, Milford, CT, USA) 3 m long and 0.25 mm in diameter was used, its granularity being 1.58 μm. ABTS solution system control parameters: column temperature circa 50°C, mobile phase flow rate 0.5 ml/min[15,16].

Sample color change in mixture with ABTS reagent after reaction ending was recorded using Waters 2487 UV/VIS detector (Waters Corporation) at wavelength of 650 nm.

In selection of analysis terms we were guided by the signal value expressed in negative peak height as a sensitivity indicator. The antioxidant potential of tested samples was determined by the comparison with that of Trolox standard solution in eight different concentrations within the range of 0.625–80 mg/ml. The constructed calibration plot was expressed with the following quadratic equation:

$Y= -1.54 \cdot 10^2X^2 + 4.16 \cdot 4.16 \cdot 10^4x - 2.08 \cdot 10^4$; R^2 (ABTS) = 0.9991.

Antioxidant potential of extracts (X, mg/g) was calculated by formula:

$$X = \frac{m_0 \cdot 20000}{m_1 \cdot (100 - w)},$$

where m_0 – mass of Trolox standard sample, g; m_1 – mass of tested sample, g; w – drying loss, wt %[14, 15].

RESULTS AND DISCUSSION

The results of our study enabled the identification of six phenolic compounds in *R. sachalinensis* herbal parts: phenolcarbonic gallic acid, two hydroxycinnamic acids (neochlorogenic, *trans*-cinnamic) and three flavonoids (rutin, hyperoside and isoquercitrin). In roots of this plant we found only gallic acid and 6,7-dihydroisoflavone. HPLC chromatograms of phenolic compounds in *R. sachalinensis* herbal parts and roots are shown in Fig. 1-2.

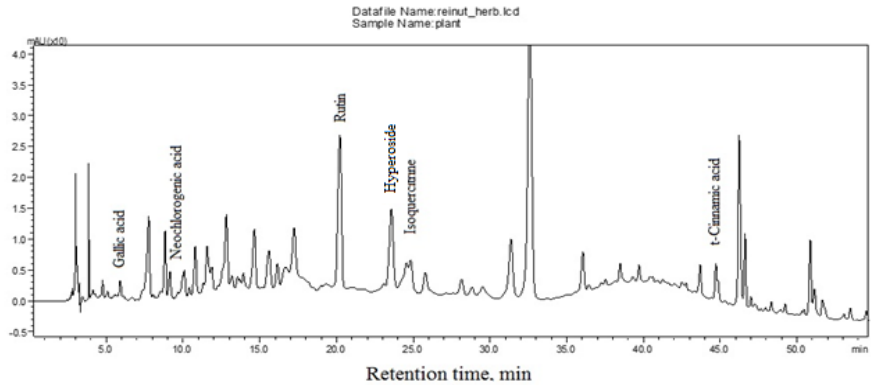

Figure 1. HPLC chromatogram of phenolic compounds in *R. sachalinensis* herbal parts

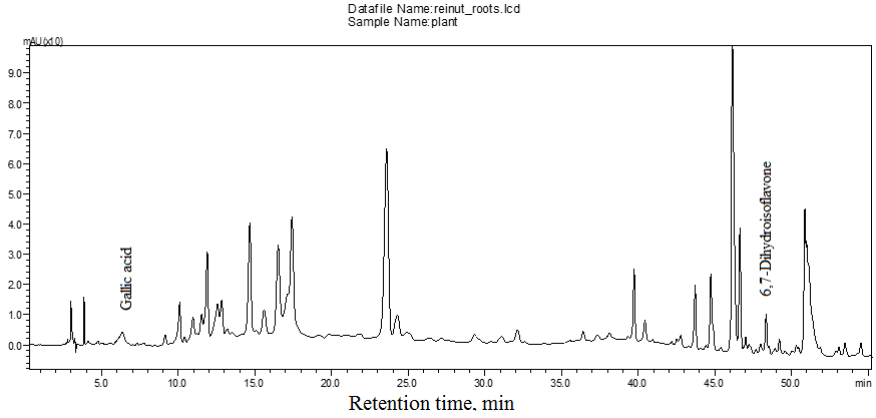

Figure 2: HPLC chromatogram of phenolic compounds in *R. sachalinensis* roots

The qualitative composition and quantitative content of phenolic compounds in *R. sachalinensis* herbal parts and roots are presented in Table 1.

Table 1. Qualitative composition and quantitative content of phenolic compounds of *R. sachalinensis* raw materials

Compound	Herbal parts		Roots	
	Retention time, min	Quantitative content, mg/kg	Retention time, min	Quantitative content, mg/kg
Phenolic acids				
Gallic acid	5.91	21.99 ± 0.55	5.91	6.98 ± 0.17
Total content of phenolic acids	—	21.99 ± 0.55	—	6.98 ± 0.17
Cinnamic acids				
Neochlorogenic acid	8.84	407.17 ± 8.56	—	—
trans -Cinnamic acid	44.72	23.39 ± 0.47	—	—
Total content of cinnamic acids	—	430.56 ± 9.90	—	—
Flavonoids				
Rutin	20.52	189.72 ± 4.55	—	—
Hyperoside	24.55	115.61 ± 2.54	—	—
Isoquercitrin	24.83	127.49 ± 2.93	—	—
6,7-Dihydroisoflavone	—	—	47.99	10.32 ± 0.24
Total content of flavonoids	—	432.82 ± 10.82	—	10.32 ± 0.24
Total content of identified compounds	—	885.37 ± 21.25	—	17.30 ± 0.43

Results are expressed as means ± SD of three measurements; $p<0.05$; «—» - not identified

The total content of identified compounds in *R. sachalinensis* herbal parts was 885.37±21.25 mg/kg. The amounts of hydroxycinnamic acids and flavonoids in this plant: 430.56±9.90 and 432.82±10.82 mg/kg respectively. Gallic acid (21.99±0.55 mg/kg) accounted for circa 2.5 % of the total content of identified compounds.

Neochlorogenic acid and rutin dominated in *R. sachalinensis* herbal parts. This raw material contained 407.17±8.56 mg/kg chlorogenic acid, 189.72±4.55 mg/kg rutin, 115.61±2.54 mg/kg hyperoside and 127.49±2.93 mg/kg isoquercitrin.

The total content of identified phenolic compounds in *R. sachalinensis* roots was 17.30±0.43 mg/kg. Gallic acid content in this part of plant was 3.3 times less than in its herbal parts. 6,7-dihydroisoflavone (10.32±0.24 mg/kg) accounted for almost 60% of the sum of identified compounds.

Antioxidant activity of bioactive substances in *R. sachalinensis* herbal parts and roots were studied *in vitro* by HPLC method in Trolox equivalent. Antioxidant activity chromatograms of *R. sachalinensis* herbal parts are shown in Fig. 3, that of the roots in Fig. 4.

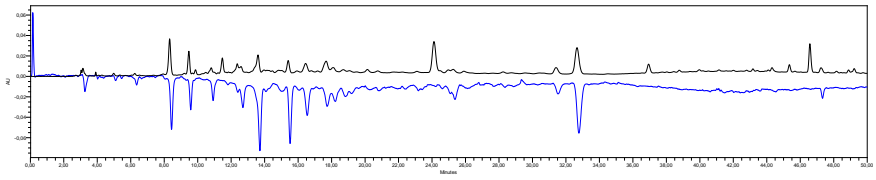

Figure 3: HPLC chromatogram of antioxidant activity determoination of *R. sachalinensis* herbal parts

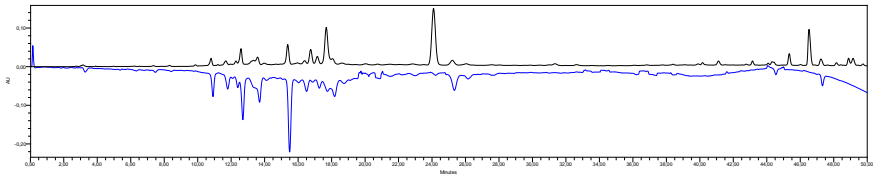

Figure 4: HPLC chromatogram of antioxidant activity determibation in *R. sachalinensis* roots

The experimental results are presented in Table 2.

Table 2. Antioxidant activity of BASs in *R. sachalinensis* raw materials

Extracts	Antioxidant capacity in Trolox equivalent, mg/g
Herbal part of extract	3.85±0.09
Roots	3.59±0.09

Results are expressed as means ± SD of three measurements; $p<0.05$.

The obtained data showed that antioxidant activities of bioactive substances in *R. sachalinensis* herbal parts and roots were in close proximity and made 3.85±0.09 and 3.59±0.09 mg/kg respectively.

Our research proved much higher versatility of phenolic compounds composition in *R. sachalinensis* herbal parts as compared to its roots. The quantitative content of those bioactive substances was much higher in *R. sachalinensis* herbal parts. The study of antioxidant activity confirmed that the antioxidant potential of both parts of tested plant was almost at an identical level. The obtained data enable our deeper knowledge of *R. sachalinensis* chemical composition and pharmacological activity and confirm the feasibility of drugs devel-

opment on the basis of *R. sachalinensis* raw materials, including drugs with antioxidant activity. Besides, this information will be useful in the development of quality control methods for *R. sachalinensis* raw materials.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Širka VH, Lakušić D, Šinžar-Sekulić J, Nikolić T, Jovanović S. Reynoutria sachalinensis: a new invasive species to the flora of Serbia and its distribution in SE Europe. Bot Serb, **2013;** 37(2): 105-112.

2. Alrikabi AYa, Tartynska HS, Zhuravel IO. A study in elementary composition of Sakhalin knotweed (Reynoutria sachalinensis (F. Schmidt) Nakai) herb. Fitoterapiia. A Journal, **2019;** 1: 70-72. – in Ukrainian. https://doi.org/10.33617/2522-9680-2019-1-70

3. Jin W, Na MK, Song GY, Lee YM, Bae KH. Cytotoxic anthraquinones and stilbenes from Reynoutria sachalinensis (Fr. Schm.) Nakai. Korean Journal of Medicinal Crop Science, **2005;** 13(2): 80-84.

4. Patocka J, Navratilova Z, Ovando M. Biologically active compounds of Knotweed (Reynoutria spp.). Mil Med Sci Lett (Voj. Zdrav. Listy), **2017;** 86(1): 17-31.

5. Nawrot-Hadzik I; Ślusarczyk S; Granica S; Hadzik J; Matkowski A. Phytochemical Diversity in Rhizomes of Three Reynoutria Species and their Antioxidant Activity Correlations Elucidated by LC-ESI-MS/MS Analysis. Molecules, **2019;** 24(6): 1136. https://doi.org/10.3390/molecules24061136

6. Nawrot-Hadzik I, Hadzik J, Fleischer M, Choromańska A, Sterczała B, Kubasiewicz-Ross P, et al. Chemical composition of East Asian invasive Knotweeds, their cytotoxicity and antimicrobial efficacy against cariogenic pathogens: an in-vitro study. Med Sci Monit, **2019;** 25: 3279-3287. https://doi.org/10.12659/MSM.913855

7. Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol, **2009;** 7(1): 65-74. https://doi.org/10.2174/157015909787602823

8. Gupta RK, Patel AK, Shah N, Choudhary AK, Jha UK, Yadav UC, et al. Oxidative stress and antioxidants in disease and cancer: a review. Asian Pac J Cancer Prev, **2014;** 15: 4405-4409. https://doi.org/10.7314/apjcp.2014.15.11.4405

9. Vaziri ND, Rodríguez-Iturbe B. Mechanisms of disease: oxidative stress and inflammation in the pathogenesis of hypertension. Nat Clin Pract Nephrol, **2006;** 2: 582-593. https://doi.org/10.1038/ncpneph0283

10. Pohanka M. Alzheimer´s disease and oxidative stress: a review. Curr Med Chem, **2014;** 21(3): 356-364. https://doi.org/10.2174/09298673113206660258

11. Kruk J, Aboul-Enein HY, Kładna A, Bowser JE. Oxidative stress in biological systems and its relation with pathophysiological functions: the effect of physical activity on cellular redox homeostasis. Free Radic Res, **2019;** 53(5): 497-521. https://doi.org/10.1080/10715762.2019.1612059

12. Pohanka M. Role of oxidative stress in infectious diseases. A review. Folia Microbiol, **2013;** 58: 503-513. https://doi.org/10.1007/s12223-013-0239-5

13. Zhang X, Thuong PT, Jin WY, Su N.D, Sok DE, Bae KH, et al. Antioxidant activity of anthraquinones and flavonoids from flower of Reynoutria sachalinensis. Arch Pharm Res, **2005;** 28(1): 22-27. https://doi.org/10.1007/BF02975130

14. Glushchenko A, Bezruk I, Ivanauskas L, Georgiyants V. Development of the method of simultaneous quantitative determination of loratadine and auxuilary substances in the combined syrup «Loratadin+». ScienceRise: Pharmaceutical Science, **2019;** 2(18): 39-47. https://doi.org/10.15587/2519-4852.2019.169511

15. Raudonis R., Raudone L, Jakstas V, Janulis V. Comparative evaluation of post-column free radical scavenging and ferric reducing antioxidant power assays for screening of antioxidants in strawberries. J Chromatogr *A,* **2012;** 1233: 8-15. https://doi.org/10.1016/j.chroma.2012.02.019

16. Raudonis R, Jakštas V, Burdulis D, Benetis R., Janulis V. Investigation of contribution of individual constituents to antioxidant activity in herbal drugs using postcolumn HPLC method. Medicina, **2009;** 45(5): 382-394.