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SHORT COMMUNICATION

ANTI-AGING COMPOUNDS IN LATVIAN WILD GROWING PLANT OF *FALLOPIA JAPONICA*

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Abstract

Fallopia japonica (Japanese knotweed) native to Japan is an aggressively invasive plant in the world, but also it contains a biological active natural compounds. Most important compounds of anti-aging by which can activate responsible gene sirtuin in nature is stilbenes *trans*-resveratrol (*trans*-3,4′,5-trihydroxystilbene), *trans*-piceid (resveratrol-3-β-mono-D-glucoside), flavonoid – butein (1-(2,4-dihydroxyphenyl)-3-(3,4-dihydroxy- phenyl)-2-propen-1-one), flavones – fisetin (3,3′,4′,7-tetrahydroxyflavone, 5-deoxyquercetin) and quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one). The aim of this study was to determine the anti-aging compounds in Latvian wild growing plant roots of *Fallopia japonica*. Before extraction roots of *Fallopia japonica* were convective dried at 60 °C. For the extraction of the anti-aging compounds from *Fallopia japonica* roots the ethanol / water solution with the concentration 70% (v/v) was used. High performance liquid chromatography (HPLC) method was used for analysis of anti-aging compounds: resveratrol, piceid, butein, fisetin, and quercetin. The obtained results showed, that the main anti-aging compounds detected by HPLC method in *Fallopia japonica* were stilbenes: resveratrol and piceid. Flavone – fisetin was detected in significantly lower concentrations than resveratrol and piceid. The remaining compounds, quercetin and butein, were detected in trace amounts.

Keywords: anti-aging, *Fallopia japonica*, trans-resveratrol, trans-piceid, HPLC.

Introduction

Along with the development of advanced technology, we are also developing a detection methods for variety of chemical compounds, as well as the identified compounds in the medical field.

The obtaining of eternal life elixir has been of great interest through human history, which has so far failed. This kind of magical substances or finding solution to this problem is extremely difficult, because the human body is a very complex life structure in which the variety of complicated biochemical transformations and processes take place and which is not completely researched. Aging is an inevitable process and it is linked to lifestyle, environmental and genetic structure (Si, Liu, 2014) and it is one of the most investigated research objects in years. One of the models, for longevity of human organisms are phytochemicals derived from plants. Phytochemicals may be from different classes: phenolic compounds, terpenes, polysulfides, quinones and polyamines (Leonov et al., 2015).

One of the phytochemicals bioactive compounds that influence the human aging, is stilbene phytoalexin *trans*-resveratrol (*trans*-3,4′,5-trihydroxystilbene) (Fig. 1), when they are infected by pathogens (bacteria, fungi) (Cademas, Packer, 2001; Stefani et al., 2007; Likhtenshtein, 2009).

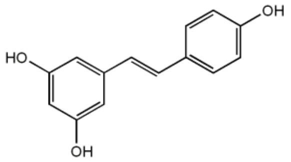

Figure 1. Chemical formula of *trans*-resveratrol

Plants are a great sources of *trans*-resveratrol and can be find in *Fallopia japonica*, red grapes, cranberry, blueberry and peanuts (Likhtenshtein, 2009).

Besides *trans*-resveratrol there is another phytochemical that influence human aging process – *trans*-piceid (polydatin, *trans*-resveratrol-3-β-mono-D-glucoside) (Fig. 2).

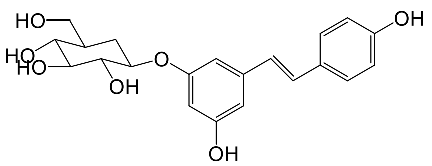

Figure 2. Chemical formula of *trans*-piceid

Trans-piceid is the glucoside of resveratrol (Fraga, 2010) that found in *Fallopia japonica*.

Third important phytochemical is butein (1-(2,4-dihydroxyphenyl)-3-(3,4-dihydroxy- phenyl)-2-propen-1-one) from flavonoid class (Fig. 3).

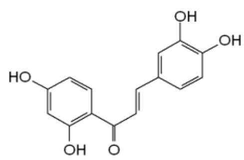

Figure 3. Chemical formula of butein

Fourth and fifth phytochemicals are two flavones: fisetin (3,3′,4′,7-tetrahydroxyflavone, 5-deoxy-quercetin) (Fig. 4) quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one) (Fig. 5)

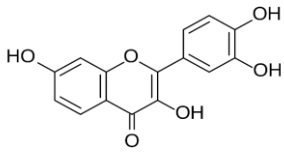

Figure 4. Chemical formula of fisetin

All five phytochemicals can increase the longevity processes of humans (see Figure 6).

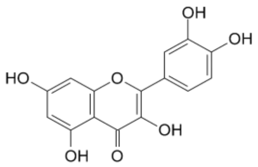

Figure 5. Chemical formula of quercetin

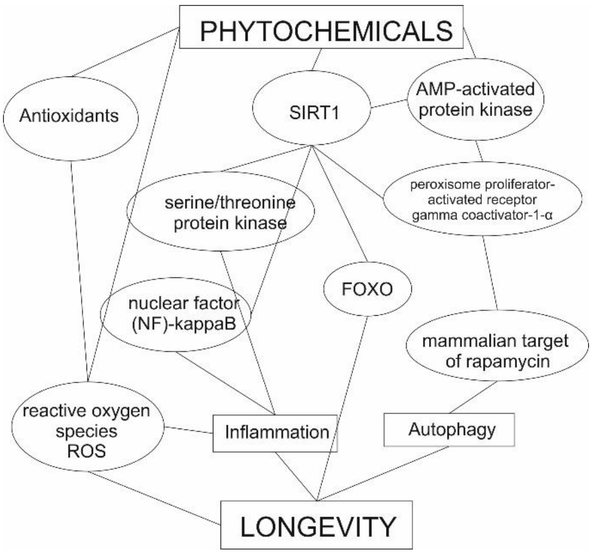

Figure 6. Increase of longevity with phytochemicals (Si, Liu, 2014)

Phytochemicals increase longevity directly or per antioxidants scavenge reactive oxygen species (ROS). Phytochemicals without ROS can activate AMP-activated protein kinase (AMPK) and SIRT1 (Sirtuin1) pathways, through inflammation and autophagy to longevity (Si, Liu, 2014). The aim of this study was to determine the anti-aging compounds in Latvian wild growing plant roots of *Fallopia japonica*.

Materials and Methods

Research was carried out at the Department of Chemistry, Faculty of Food Technology at the Latvia University of Agriculture. The object of the research was roots of *Fallopia japonica*.

Reagents

For research HPLC grade chemical reagents were used after filtration through a 0.45-µm pore size membrane filter. Ultrapure water was used in all work. *Trans*-resveratrol, *trans*-piceid, butein, fisetin, and quercetin was obtained from Sigma-Aldrich.

Preparation and extraction of samples

Roots of *Fallopia japonica* were harvested in the end of December of 2017 from Ogre district. Roots were convective dried for 24 hours at temperature +60 °C. After drying sample was ground n a laboratory mill and fitted through a 0.2 mm sieve. Powder of roots of *Fallopia japonica* 2.5 g was extracted on the magnetic stirrer with 11.0 mL of ethanol / water solution with the concentration 70% (v / v) for 60 min. Extract was centrifuged at 13 000 rpm in a centrifuge for 5 min, the supernant was then filtered through 0.45 µm pore size membrane filter and were kept at temperature of -18 °C until HPLC analysis.

Detection of trans-resveratrol, trans-piceid, butein, fisetin and quercetin with liquid chromatography

Content of *trans*-resveratrol, *trans*-piceid, butein, fisetin and quercetin was determined by high performance liquid chromatography (HPLC) (Schimadzu LC-20 Prominence, Shimadzu USA Manufacturing Inc, Canby, USA), detector DAD SPD-M20A, Solvent Delivery Unit LC-20AD, Column Oven CTO-20A, Autosampler SIL-20A, System Controller CBM-20A and data system LCsolution software.

Preparation of calibration solution

Weight in 50 mL volumetric flask with narrow neck 5.8±0.1 mg *trans*-piceid, 4.9±0.1 mg *trans*-resveratrol, 2.0±0.1 mg butein, 6.0±0.1 mg fisetin, 5.6±0.1 mg quercetin and fill with ethanol till mark and mix. Calibration chromatograms of calibration solution are given in Figures 7, 8 and 9.

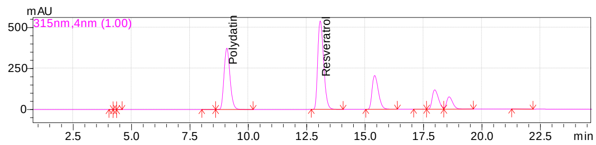

Figure 7. Calibration chromatograms of *trans*-piceid and *trans*-resveratrol

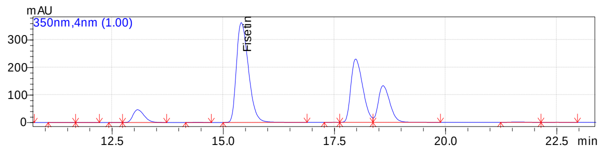

Figure 8. Calibration chromatograms of fisetin

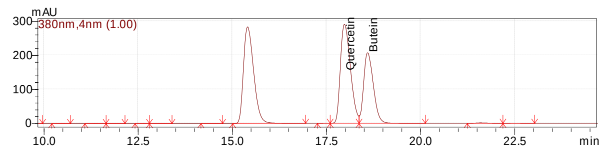

Figure 9. Calibration chromatograms of quercetin and butein

Parameters of chromatography: the analytical column YMC C18, 4.6 mm x 250 mm, 5 µm and temperature of column +30 °C were used for separation. Wavelength 315 nm for *trans*-piceid, *trans*-resveratrol, 350 nm for fisetin and 380 nm for butein, quercetin. Injection volume of sample 1 µL for *trans*-piceid, *trans*-resveratrol, 10 µL for fisetin, butein, and quercetin. Mobile phase: A (*deionized water), B (*HPLC grade *CHROMASOLV*® methanol) and C (Acetic acid solution for HPLC) in the gradient conditions was used. Flow rate was 0.8 mL min^{-1}. Gradient conditions: start B (35 mL), C (2 mL); 15 min. B (55 mL), C (8 mL); 20 min. (55 mL), C (8 mL); 21 min. B (35 mL), C (2 mL); 30 min – stop.

Statistical analysis

The results were processed by mathematical and statistical methods (mean, standard deviation) using Microsoft Office Excel 2016.

Results and Discussion

The obtained results of research showed, that the main anti-aging compounds detected by HPLC method in *Fallopia japonica* were stilbenes: *trans*-resveratrol and *trans*-piceid (Fig. 10).

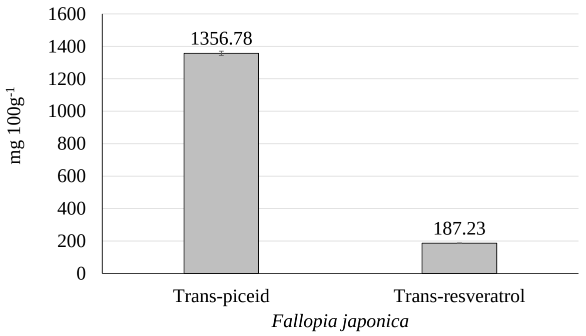

Figure 10. Content of *trans*-piceid and *trans*-resveratrol

In roots powder *trans*-piceid content was very high 1356.78 mg 100 g^{-1} comparing to other anti-aging compounds in the sample. *Trans*-resveratrol content 187.23 mg 100 g^{-1} was approximately seven times lower than *trans*-piceid. Another results showed that content of both substances can be in range from 670 to 1220 mg 100 g^{-1} for *trans*-piceid and 104 to 390 mg 100 g^{-1} for *trans*-resveratrol (Lin et al., 2016; Zhang et al., 2015; Jin et al., 2013). *Trans*-piceid is a glucoside and it is natural precursor of *trans*-resveratrol (De Maria et al., 2013). *Trans*-piceid is metabolized in the small intestine of human body to form of *trans*-resveratrol (Fig. 11) (Wang et al., 2013).

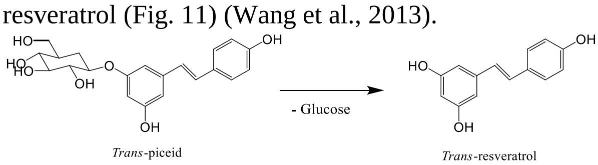

Figure 11. Biotransformation of *trans*-piceid to *trans*-resveratrol (Wang et al., 2013)

Besides *trans*-piceid and *trans*-resveratrol in plant root fisetin was detected in significantly lower concentrations. The remaining compounds, quercetin and butein, were detected in trace amounts (Fig. 12).

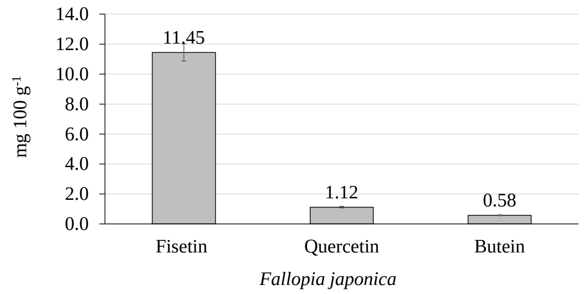

Figure 12. Content of fisetin, quercetin and butein

Fisetin content was 11.45 mg 100 g^{-1}, quercetin 1.12 mg 100 g^{-1} and butein 0.58 mg 100 g^{-1}, respectively

Conclusions

In research it was detected that the main anti-aging compound phytochemicals in extract of *Fallopia japonica* roots were *trans*-piceid and *trans*-resveratrol and those are good source for activating Sirtuin gene that increase longevity.

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