BIOACTIVE SUBSTANCES FROM INVASIVE KNOTWEED SPECIES

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Abstract: *Knotweed is in Europe and America a highly invasive plant, originating from Asia. Three different taxons are identified in Europe, namely Japanese knotweed (Fallopia japonica), Sakhalin knotweed (Fallopia sachalinensis) and Bohemian knotweed (Fallopia x bohemica). The best-known among them is F. japonica that has been since ancient times used in traditional Chinese medicine for treatment of different kind of diseases (e.g. inflammatory diseases, hepatitis, tumors, burns and hyperlipidemia). Several studies have recently been performed to confirm that the Japanese knotweed extract possess several different bioactivities, namely antioxidant, antidiabetic, antiviral, antibacterial, and antimycotic activity. It was shown that many different bioactive components are responsible for this. However, much less studies have been performed on the other two knotweed species, particularly on the Bohemian knotweed, which is the most widely spread due to its highest invasiveness among the three taxons. The results of our study confirmed antioxidant and antimicrobial activities of extracts obtained from different tissues (rhizomes, leaves, stalks and flowers) of all three knotweed species.*

Keywords: *knotweeds, antioxidants, antimicrobial and antidiabetic activity.*

1. Introduction

It is well established that variety in food is important to provide essential nutrients in the human diet. Oxidative stress is a major induction factor in many chronic and degenerative diseases [1]. Antioxidants, which allow the organism to defend against oxidative stress, environmental pollution and other toxic insults, play an important role in the healthy human nutrition. A great number of plants contain chemical compounds exhibiting antioxidant properties as well as antimicrobial activity. For that reason, they can be used in food industries to prevent food degradation and alteration and to minimize the undesirable effects of synthetic food preservatives in human health. In addition to plant foods, the nutrition recommendations are suggesting to consume a variety of food supplements based on plant extracts to avoid synthetic medications.

2. Knotweed in traditional medicine

Knotweed is in Europe and America a highly invasive plant, originating from Asia. Three different taxons are identified in Europe, namely Japanese knotweed (*Fallopia japonica*), Sakhalin knotweed (*Fallopia sachalinensis*) and Bohemian knotweed (*Fallopia x bohemica*), the hybrid of these two species. All three species are often only referred as a source of environmental and social problems, but in some parts of the world these plants have an important role in maintenance of human health and are included in the everyday human diet [2].

The best-known among them is *F. japonica* that has been since ancient times used in traditional Chinese medicine for treatment of different kind of diseases (e.g. inflammatory diseases, hepatitis, tumors, burns and hyperlipidemia). Among tissues, the most commonly used are rhizomes for reducing fever, treatments of high blood pressure and body detoxification. Dried stems are used in alternative medicine for treatment of inflammatory diseases, hepatitis and diarrhea. Furthermore, in China and Japan, Itadori tea is a traditional herbal infusion used for treating heart disease [3].

3. Recent studies of knotweed bioactivities

Several studies have recently been performed to confirm that the Japanese knotweed extract possess several different bioactivities, namely antioxidant, antidiabetic, antiviral, antibacterial, and antimycotic activity [3-12]. It was shown that many different bioactive components are responsible for this, among which stilbenes (e.g. emodin, resveratrol and resveratrol derivatives), anthraquinones, flavonoids (e.g. catechin, epicatechin) and lignins are the most predominant. Resveratrol, one of the most powerful antioxidant and recently also among the most studied polyphenols, is a naturally occurring polyphenol typically associated with grapes and red wine, but also abundantly present in knotweed. There are already a few supplements on the market with resveratrol originating from the rhizomes of Japanese knotweed.

It has been also shown that different tissues of all three taxons contain several other polyphenols, e.g. polydatin, quercetin, and epicatechin, which besides antioxidant properties possess significant antimicrobial and anti-inflammatory activities. It is therefore expectable that these plants will be in the future used for the development of new functional foods and for the isolation of novel food ingredients and pharmaceutical molecules. So far, research in the study of secondary metabolites have mostly focused on Japanese knotweed and its rhizome. In the future, it would be important to accurately analyse also the components of the aboveground parts of these invasive plants and special attention must be given to the other two taxons.

3.1. Polyphenols and antioxidant capacity

In our study [5], different tissues (rhizome peels, rhizomes, leaves, stalks and flowers) of all three knotweeds were freeze-dried and extracted with 50% ethanol. The presence of resveratrol, polydatin, (+)-catechin and (-)-epicatechin was detected by a HPLC/DAD system, the content of each was quantified and expressed as mass per gram of freeze-dried tissue. It can be seen from Figure 1 to Figure 4 that content of all determined representatives of polyphenols depend not only on taxon, but also on the tissue of the particular knotweed. Bohemian knotweed flower is the richest source of resveratrol (476 μg/g), while Japanese knotweed rhizome peels are the richest source of polydatine (23.3 mg/g). The richest source of (+)-catechin (13.3 mg/g) is Sakhalin knotweed rhizome, whereas (-)-epicatechin (27.1 mg/g) is the most abundantly present in flowers of Bohemian knotweed.

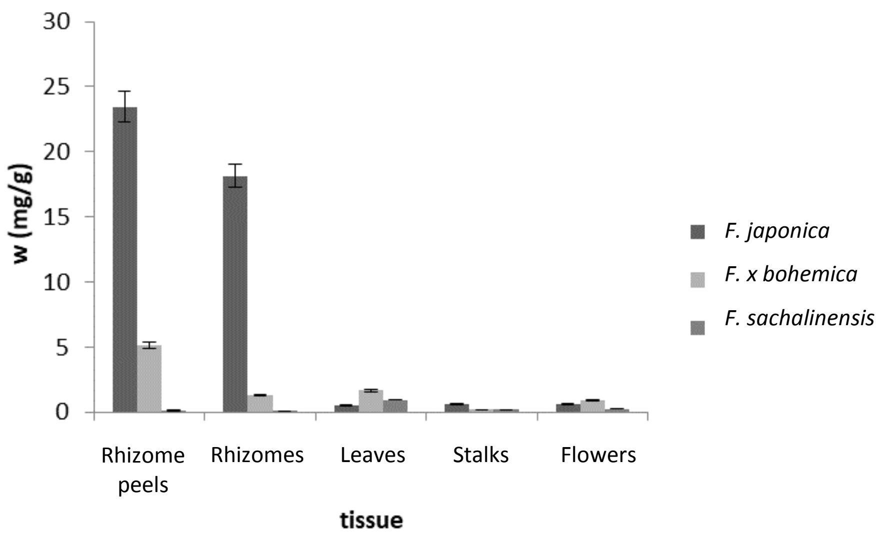

Fig. 1. *Mass fraction (w) of polydatin in different tissues of three knotweed species*

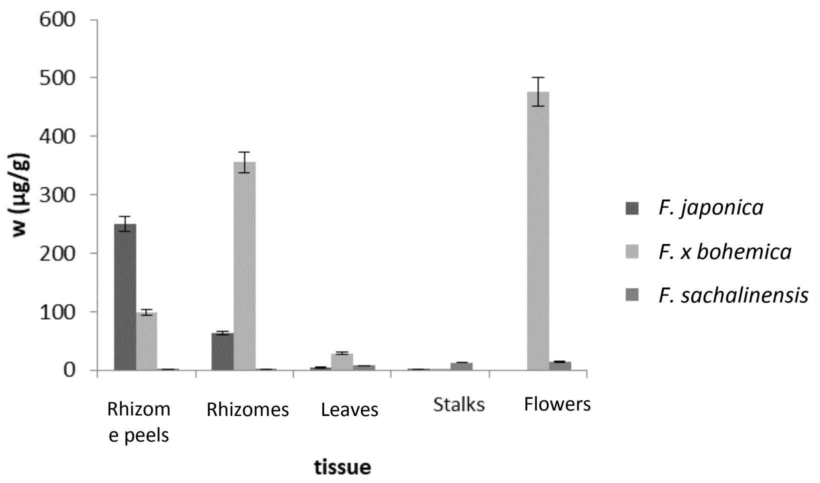

Fig. 2. *Mass fraction (w) of resveratrol in different tissues of three knotweed species*

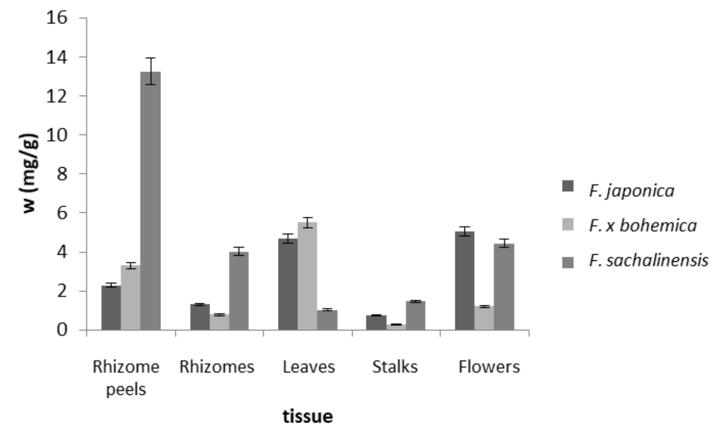

Fig. 3. *Mass fraction (w) of (+)-catechin in different tissues of three knotweed species*

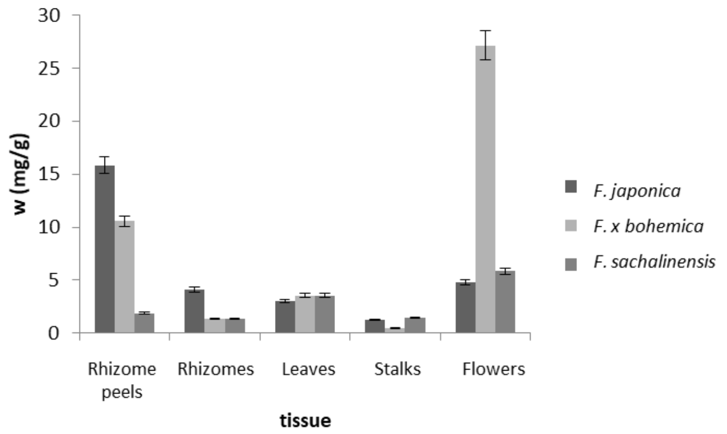

Fig. 4. *Mass fraction (w) of (-)-epicatechin in different tissues of three knotweed species*

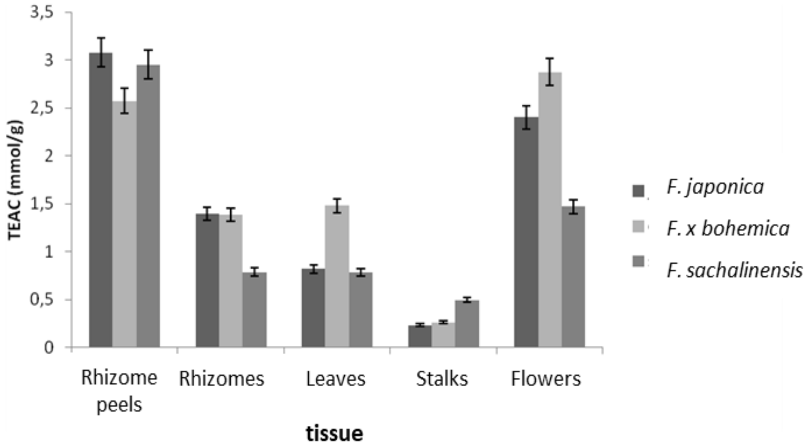

Fig. 5. *Trolox equivalent antioxidant capacity (TEAC) in different tissues of three knotweed species*

Antioxidant capacity was determined with ABTS• assay and results were expressed as trolox equivalent antioxidant capacity (TEAC) per gram of freeze-dried tissue (Figure 5). Similarly to the above results, the highest TEAC was determined in rhizome peels and in flowers of all three taxons. The highest TEAC among tested extracts was determined in rhizome peels of Japanese knotweed (3.0 mmol/g), while the lowest was detected in stalks of Japanese knotweed (0.23 mmol/g).

3.2. Antimicrobial activity

In order to determine antimicrobial activity of ethanol extracts of rhizome peels obtained from all three taxons, the tissues were freeze-dried and re-dissolved in less ethanol to obtain the concentration of dry extract equal to 600 mg/mL. The antimictobial activity of concentrated extracts was evaluated with gram-positive bacteria *Listeria monocytogenes*, gram-negative bacteria *Escherichia coli* and yeast *Candida albicans*. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extracts were determined by combination of broth microdilution and plate-count methods [6]. The highest antimicrobial activity of the knotweed extracts among tested microorganisms was observed on the gram-positive bacteria *L. monocytogenes*. The Japanese knotweed extract was bacteriocidal at a concentration of 12 mg/mL, whereas the extracts of Sakhalin and Bohemian knotweed at a concentration of 23 mg/mL. Gram-negative bacteria *E. coli* was more resistant, since all the extracts were inhibiting its growth at a concentration of 19 mg/mL, and a Japanese knotweed extract with concentration 75 mg/mL and more was bacteriocidal. The most resistant among tested microorganisms was the yeast *C. albicans*, since it its growth was inhibited by Japanese and Bohemian knotweed extracts only at concentration of 75 mg/mL and 37 mg/mL extract of the Sakhalin knotweed.

Japanese knotweed was the only taxon with bactericidal activity against *E. coli* (75 mg/mL). Also, at a concentration of 12 mg/mL, bactericidal activity of the same extract was observed on bacteria of *L. monocytogenes*. Sakhalin knotweed extract with concentration of 37 mg/mL was the most effective in inhibition of yeast *C. albicans.* Regarding Bohemian knotweed rhizome extract, the bacterial growth was inhibited at the same concentrations as the Sakhalin knotweed extract, namely bacteria *E. coli* was inhibited at a concentration of 19 mg/mL and *L. monocytogenes* at a concentration of 23 mg/mL. The yeasts *C. albicans* where the extract of the Sahalinsk hauler was more effective. It was also found that knotweed taxons with higher TEAC had higher inhibition and/or bacteriocidal activity against selected microbial strains.

4. Conclusions

The results of our recent studies confirmed antioxidant and antimicrobial activities of extracts obtained from different tissues (rhizomes, leaves, stalks and flowers) of all three knotweed species. It has to be stressed that the previous studies have mostly been performed on Japanese knotweed, so analyzing Bohemian knotweed, which is the most widely spread due to its highest invasiveness among the three species, has a very big importance for the future. The lack of studies on this taxon can be attributed to the fact that this species was created by the natural crossing of the other two taxons in Europe and appeared much later than the other two and it was many times also misclassified as Japanese knotweed. Based on our analysis, further research would be a great challenge and motivation to find other possible bioactive substances besides the ones identified in our study and to study their bioactive effects

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