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## Screening of the chemical content of several *Limniris* group Irises

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### ABSTRACT

This article puts together results from several preliminary testing on eight *Iris*, group *Limniris*, species. We used simple testing methods to prove the absence or presence of twelve chemical compounds (flavonoids, phenols, quinones, tannins, saponins, cardiac glycosides, terpenoids, alkaloids, steroids, glycosides, proteins) in samples from rhizomes, leaves and flowers of both botanical species and cultural hybrids. Together we used twenty five samples from the years 2012 and 2013. The best result, the highest amount of tested chemicals was in *Iris pseudacorus* 'Roy Davidson', rhizome, 2013 and the worst result, the lowest amount of tested chemicals was in *Iris pseudacorus*, also the year 2013, but this sample was made from the flowers of wild species.

**Keywords:** *Iris*, drop tests, chemical content, *Limniris*.

### 1. Introduction

Genus *Iris* contains around 300 species which are widely distributed across the Northern Hemisphere. Irises are mainly used as the ornamental plants due to their colorful flowers, but a lot of *Iris* species were also used in many parts of the world as medicinal plants for healing a wide spectrum of diseases. Nowadays botanical and biochemical research brings new knowledge about chemical compounds in roots, leaves and flowers of the *Iris* species, about their chemical content and possible medicinal usage. The research of Kukulka-Koch states in their review of 122 chemical compounds that were confirmed in Irises, which makes these plants very rich in secondary metabolites [7].

### 2. Material and Methods

As the research material, twenty five samples prepared from rhizomes, leaves and flowers of eight *Iris* species (*I. crocea*, *I. ensata*, *I. orientalis*, *I. pseudacorus*, by which was also used cultivar 'Roy Davidson', *I. setosa*, *I. sibirica* with cultivars 'Supernatural' and 'Whiskey White', *I. spuria* and *I. versicolor*) were used. Most of the samples were freshly prepared in the year 2013 but we also used some rhizome samples from 2012 to valorize a possible increase or decrease of chemical content depending on time.

All plants were grown for the last 6 years in the termophytic conditions of central Europe. Plants were growing in rows oriented in the east-west directory, under full sun conditions, but with an irrigation system to provide a good amount of water. The plant material was taken in the flowering season.

All parts of the plant (leaves, rhizomes and flowers) were dried at room temperature (22–24 °C). Dried material was homogenized on mechanical mill IKA MF10 basic (sieve 2 mm, speed 500 rpm). The homogenized material was watered with 75% methanol, and left unattended for 24 hours. The solution was filtered and storage in fridge by –4 °C. This basic solution was then used in all the tests.

For the testing were used so called "drop tests". Their benefits are that just a small amount of solution and chemicals are used, quick evaluation, usage of common chemicals, easy preparation and usage of the same solution for all tests. A disadvantage is inaccurate assessment; it is difficult to know the exact content of the compound. The tested compounds were:

**Flavonoids** (in the result table marked as FLA) - 1 ml of extract and a few drops of diluted sodium hydroxide (NaOH) added. An intense yellow colour was produced in the plant extract which becomes colourless with the addition of a few drops of diluted acid indicates the presence of the flavonoid.

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**Phenols** (in the result table marked as PHE) – 1 ml of extract and 2 ml of distilled water were added, followed by a few drops of 10% ferric chloride (FeCl<sub>3</sub>). Appearance of blue or green color indicates presence of phenols

**Quinones** (in the result table marked as QIN) – 1 ml of extract and 1 ml of concentrated sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) was added. Formation of red color shows the presence of quinones

**Tannins** (in the result table marked as TAN) - 2 ml of 5% ferric chloride added to solvent free extract. The presence of tannin is indicated by the formation of bluish black or greenish black precipitate.

**Saponins** (in the result table marked as SAP) – 2 ml of extract, 20 ml of distilled water was added and shaken vigorously in warm conditions. The formation of a honey comb-like foam indicates the presence of saponins.

**Cardiac glycosides** (in the result table marked as C. GLY) - 5 ml of extract was treated with 2 ml of glacial acetic acid containing a drop of ferric chloride (FeCl<sub>3</sub>) solution. Afterwards it was underplayed with 1 ml of concentrated sulphuric acid (H<sub>2</sub>SO<sub>4</sub>). A brown ring of the interface indicates a deoxy sugar characteristic of cardenolites.

**Terpenoids** (in the result table marked as TER) - 5 ml of each extract was mixed with 2 ml of chloroform. 3 ml of concentrated sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) was then added to form a layer. A reddish brown precipitate coloration at the interface formed indicated the presence of terpenoids.

**Alkaloids** (in the result table marked as ALK) – 3 ml of the extract, 3 ml of 1 % HCl was added with continuous stirring on the steam bath. To the mixture Mayer's reagent and Wagner's reagent were added. Formation of turbidity in the resulting precipitate indicates the presence of alkaloids.

**Steroids** (in the result table marked as STE) – 2 ml of extract was dissolved in 2 ml of chloroform and 2 ml concentrate sulphuric acid in a test tube, a red color produced in the lower chloroform layer indicates presence of steroids.

**Glycoside** (in the result table marked as GLY) – 2 ml of extract was dissolved in 2 ml of chloroform, where 2 ml of acetic acid was carefully added. A color change from violet/blue to green indicates the presence of the steroidal ring (i.e. a glycine portion of glycoside).

**Proteins** (in the result table marked as PRO) – 2 ml of extract was treated with one drop of 2% copper sulphate solution. To this 1 ml of ethanol (95%) was added, followed by an excess of potassium hydroxide pellets. A pink color in the ethanolic layer indicated the presence of proteins

### 3. Results

Complete results from all the tests are in Table 1. Reactions were evaluated on a scale with six values. The best results, strongest reaction, are marked with four plus marks (++++), samples without any reaction are marked with two minus marks (- -). Other reactions are marked as follows: weak uncompleted reaction (-), weak reaction (+), strong reaction,

but with some deficiencies in coloration (++) , strong reaction (+++). From the results we can see that the best result, the richest reactions were in the sample *I. pseudacorus* 'Roy Davidson', rhizome from 2013. The weakest reaction, the lowest content of researched chemicals has *I. pseudacorus*, flower from 2013.

In the test for steroids the reaction with sulphuric acid was too strong and due to that, we did not obtain any significant results.

### 4. Discussion

Flavonoids were confirmed in rhizomes, leaves and flowers of *Iris nigricans* [5]. Also the glycoflavones and isoflavones in *Iris tenuifolia* were proved in the laboratory [18]. These results were also confirmed by the research of other authors [1]. Although *Iris nigricans* belongs to the section *Oncocyclus* [9], it is still part of the genus *Iris* so we can assume similar results by some other plants from this genus. Flavonoids, isoflavonoides and their glycosides, benzoquinones and terpenoids are mentioned as chemicals largely emerging in the genus *Iris* [6]. Positive proof of flavonoids was confirmed also in *Iris spuria* [12]. The presence of isoflavonoides in the leaves of many bearded irises (*I. pallida*, *I. germanica*, *I. variegata*, *I. albicans* etc.) was also proven by Williams [16]. In our research the presence of flavonoids was confirmed in 16 samples, all from the year 2013. The best reaction (+++) was in samples from rhizomes of *I. sibirica* 'Whiskey White', *I. spuria*, *I. setosa*, *I. versicolor*, in leaves of *I. spuria*, *I. pseudacorus* and *I. pseudacorus* 'Roy Davidson' and in flowers of *I. sibirica* 'Whiskey White' and flowers of *I. pseudacorus* 'Roy Davidson'. Three samples which did not have any reaction for flavonoids (--) were rhizome from *I. ensata* from year 2012, leaf from *I. setosa* and flower from *I. sibirica* 'Supernatural' from 2013.

Phenolic compounds were proved in flowers and leaves of Japanese iris species, *I. rossii*, which belong to the *Limniris* group. The author of the article also mentioned the works of other authors which proved presence of phenols in *I. japonica*, *I. pseudacorus*, *I. gracilipes*, *I. setosa*, *I. laevigata* and *I. ensata* [8]. Even in the seeds of *I. pallasii*, (*Limniris* group) seven phenolic compounds were found [11]. From our samples the best reaction (+++) was seen in samples from rhizomes of *I. spuria*, from both years (2012, 2013). Moreover, there were another six samples which have (++) and eleven samples with a (+) reaction. Only the sample from flowers of *I. pseudacorus* and *I. setosa* gave no positive reaction.

Presence of quinones is mentioned in seeds of *Iris tectorum* [13] and in *Iris bungei* [10]. In our research there was a very strong positive reaction in rhizomes of *I. pseudacorus* from both years (2012, 2013) and also in rhizomes of its cultivar 'Roy Davidson'. There were another five samples with (++) reaction and five with (+) reaction. Rhizome from *I. ensata* from 2012, leaves from *I. setosa*, *I. sibirica* 'Supernatural' and *I. spuria* gave no response in the test.

Terpenoids were confirmed as the main chemical compound of fragrance in *Iris lutescens*. The dominant part was myriscine, (E)-β-ocimene and limonen [15]. Burrows and Tyr1 states that rhizomes of *Iris missouriensis* are toxic due to high content of terpenoids and quinones [3]. *Iris missouriensis* belong to the subgenus *Limniris*, section *Limniris* [9] to which we can also include all researched plants.

Presence of tannins was confirmed in *I. versicolor* [4] which

belong to the same group as the researched *I. pseudacorus*. This congeniality was proved by our research, the strongest positive reaction (+++) for tannins was in rhizomes of *I. sibirica* 'Supernatural' and 'Whiskey White' and in rhizomes of *I. ensata* (sample from 2012), and some minor reactions (+) in another seven samples. There were just two samples with no reaction: the rhizomes of *I. crocea* from 2012 and flowers of *I. pseudacorus*.

Positive confirmation for saponins in Iridaceae brings Waller and Yamasaki in species of *Crococsmia × crocosmiiflora* and *C. masoniorum* <sup>[14]</sup>. Although this are not typical iris species other authors also states saponins as chemical compounds typical for *Iridaceae* <sup>[19]</sup>. We have just one sample with a strongly positive (+++) reaction on saponins, the rhizome of *Iris setosa*, and another four samples with a weaker (++) reaction and nine samples with a minimal (+) response.

The presence of cardiac glycosides (digoxin, digitoxin, ouabain) was mentioned in the genus *Iris*, but without any specification for genus or concrete species <sup>[18]</sup>. We have three samples with a highly positive (+++) reaction for cardiac glycosides and all of them were samples made from the rhizomes of *I. pseudacorus*. A weaker reaction (++) was

*pseudacorus* and *I. pseudacorus* 'Roy Davidson'. We also have a weaker reaction (++) in root, leaves and flower samples

observed in five samples and a minimal reaction (+) in six samples. Just the rhizome of *I. orientalis* from 2012 and leaf from *I. setosa* and *I. spuria* have no reaction at all (--).

Watson and Dallwitz mentioned in their summary of phytochemical profile in Iridaceae that the alkaloids were common <sup>[20]</sup>. The presence of alkaloids in *I. versicolor* was proven by Grieve <sup>[19]</sup>. There was no clear reaction for alkaloids, although in our research there were some minimal (+) responses in fifteen samples.

As for glycosides, there is proof for isoflavone glycosides (iriskashmirianin 49-O-b -D-glucoside, nigricin 49-O-b -D-glucoside, irilone 49-O-b -D-glucoside a iridin) in *Iris germanica* <sup>[2]</sup>. In our research there were just two samples with a minimal reaction (+) for glycosides. The sample from leaves of *I. spuria* was the only one with no reaction (--).

Although we have one sample with a highly positive reaction (+++), six positive reactions (++) and seven samples with a minimal (+) reaction we find literary proof for the presence of tannins just for the *Iris versicolor* <sup>[18]</sup>.

Table 1.

Species	Collecting year	plant part	ALK	PHE	FLA	QIN	PRO	SAP	C. GLY	GLY	TAN	TER
<i>I. crocea</i>	2012	rhizome	+	++	-	+	-	-	+	-	+	--
<i>I. ensata</i>	2012	rhizome	-	-	--	--	-	+	+++	-	--	++
<i>I. orientalis</i>	2012	rhizome	+	+	-	+	-	--	--	-	+	-
<i>I. pseudacorus</i>	2012	rhizome	-	++	-	+++	-	+	+++	-	+	+++
<i>I. pseudacorus</i>	2013	rhizome	-	++	-	+++	-	-	+++	-	+	++++
<i>I. pseudacorus</i>	2013	leaf	-	+	++	-	-	-	+	-	+	+
<i>I. pseudacorus</i>	2013	flower	+	--	+	-	-	-	-	+	--	--
<i>I. pseudacorus</i> 'Roy Davidson'	2013	rhizome	+	++	+	+++	-	-	+++	-	++	+++
<i>I. pseudacorus</i> 'Roy Davidson'	2013	leaf	-	+	++	-	-	+	-	-	++	+
<i>I. pseudacorus</i> 'Roy Davidson'	2013	flower	+	-	++	++	-	-	++	-	++	-
<i>I. setosa</i>	2013	rhizome	+	+	++	-	-	+++	-	-	-	-
<i>I. setosa</i>	2013	leaf	-	+	--	--	-	+	--	-	++	-
<i>I. setosa</i>	2013	flower	+	--	+	+	-	+	+	-	--	-
<i>I. sibirica</i> 'Supernatural'	2013	rhizome	+	+	+	++	-	-	+	-	-	++
<i>I. sibirica</i> 'Supernatural'	2013	leaf	+	-	+	--	-	++	++	-	-	++
<i>I. sibirica</i> 'Supernatural'	2013	flower	-	++	--	++	-	-	++	-	-	++
<i>I. sibirica</i> 'Whiskey White'	2013	rhizome	+	+	++	++	-	++	-	-	-	++
<i>I. sibirica</i> 'Whiskey White'	2013	leaf	+	+	-	-	-	++	-	-	-	++
<i>I. sibirica</i> 'Whiskey White'	2013	flower	-	++	++	-	-	+	-	+	++	++
<i>I. spuria</i>	2012	rhizome	+	+++	+	-	-	+	+	-	++	+
<i>I. spuria</i>	2013	rhizome	+	+++	++	+	-	-	-	-	+++	+
<i>I. spuria</i>	2013	leaf	+	-	++	--	-	++	--	--	-	-
<i>I. spuria</i>	2013	flower	-	+	+	+	-	-	++	-	+	+
<i>I. versicolor</i>	2013	rhizome	+	+	++	++	-	+	+	-	-	+
<i>I. versicolor</i>	2013	leaf	-	+	-	-	-	+	-	-	+	-

## 5. Conclusion

With this research, it was confirmed that the *Linniris* group irises are rich in chemical content and they are a promising group for further research. From findings, it was clear that it cannot be strictly determined which part of the plant has the lowest content of chemical compounds. In the flowers of *Iris pseudacorus* there was a confirmed presence of just three examined chemicals, but in flowers of *I. sibirica* 'Whisley White' were five of them. Nevertheless, it can be said that samples prepared from rhizomes have better result than samples prepared from flowers of leaves. It was also not confirmed for the change in content of researched chemicals with time. The sample prepared from rhizomes of *I. pseudacorus* from year 2012 had almost the same results as the sample from year 2013. Same situation was in rhizome samples of *I. setosa*. We can say that the best result, the highest amount of tested chemical has sample *Iris pseudacorus* 'Roy Davidson', rhizome, 2013 and the worst result, the lowest amount of tested chemicals was in *Iris pseudacorus*, also year 2013, but this sample was made from flowers.

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