DYEING POTENTIAL OF THE IRIS SIBIRICA L. FLOWERS

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Abstract

This article put together results from several experiments which led to the measuring of the color of *Iris sibirica* L. flowers and using them as dye for coloring cloth. In first phase of our experiments we use the RHS color chart to measure the color of the flowers on the plant, the HPLC DAD to compare presence of six anthocyanin colorants with the methanolic extract from the flowers. We also did several tests to prove presence of basic plant metabolites groups in the iris flowers. In second phase we used the iris flowers to dye cotton cloth. We measured the cloth color by the RHS color chart and by the Konica Minolta Spectrophotometer the day after dyeing and the RHS color chart to measure it again after 6 months. Our results show high potential for the usage of the *Iris sibirica* L. flowers as a source for cloth dyeing.

Keywords: Iris sibirica L., cloth dyeing, Konica Minolta Spectrophotometer, RHS color chart

Introduction

Genus *Iris* contains around 300 species and is divided into six subgenera with 12 sections, one of which being *Limniris* (Wilson, 2004). Plants which belong to this section can be found growing throughout the Northern Hemisphere; in forests, on the sides of mountains, along coast lines, in swamps and wet meadows, and in dry, scrubby regions (Austin, 2005).

Nowadays, about eighty species belong to the *Limniris* group which don't have the same evolutionary history. The presence of the rhizomes and absence of the „beard" are just the signs within the genus *Iris* and are not defined as an monophyletic signs of the group (Wilson, 2004).

Iris sibirica L. is the first and most typical plant from the group of Siberian irises which contain eleven species in total (Speichert and Speichert, 2004). Siberian irises can be divided into two groups according to the number of chromosomes. The first group has 28 chromosomes, the second 40 chromosomes. All used plants belong to the first group which consists of the most common and easiest-to-grow species, including the traditional blue-flowered types that are derived from Iris sanguinea Donn ex Hornem and *I. sibirica* L. (Austin, 2005). Siberian iris cultivars are excellent for a bog garden, for the edge of a stream, or for a seasonal wet spot in the backyard. In colder climates, they prefer wet soils in the spring and summer, but generally require drier conditions in the fall and winter. If they are placed in a pond in the spring, they should be removed before winter frost arrives, and mulched in the perennial border. In warmer climes, where the temperatures do not drop below -7 °C, they do not need mulch. When first offered Siberian irises were limited in color to blue and white. Now, the range has been greatly expanded to include deep purple-reds to light lavender-pinks. Flowers are anywhere from 5 to 10 cm wide, depending upon the selection. Since 1970, hybridizers have been cross-pollinating the various species in the Siberian group with *I. sibirica* L., creating hybrids whose parentage is now so complicated that the cultivars

are no longer listed with a species name. Several hundred Siberian iris cultivars are registered with the American Iris Society (Speichert and Speichert, 2004).

Materials and Method

Plants from which the flowers were obtained were grown by the 6th year in the grounds of the Horticulture Faculty in Lednice. Plants grown in the free soil, in a sunny spot, in rows oriented in an east-west direction. Plants grow in the conditions of loamy soils, in direct sunlight in rows with spacing of 70cm between plants and 50cm between rows. Spring irrigation is introduced on the experimental ground and it runs every 3 days in summer to provide enough water (3l per plant) for the plants to grow. In summer the plants also get 50 grams amount of classical NPK (15-15-15) fertilizer on each square meter of the experimental field.

Together we used 17 cultivars of *Iris sibirica* L. mostly with blue and sometimes purple flowers.

Table 1. Brief characterization of the used cultivars of *I. sibirica* L.

Cultivar name	Parents	Year of registration
Ann Dasch	´Gatineau´ x ´Dreaming Spires´ X unknown seedling	1977
Cambridge	´White Swirl´ X ´Gatineau´	1964
Dark Desire	´White Swirl´ X ´Tealwood´	1974
Dreaming Spires	´White Swirl´ X ´Tycoon´	1964
Elfelde	unknown	unknown
Friendly Welcome	´Dreaming Spires´ X ´Dark Desire´	1977
Grand Junction	´Tunkhannock´ X ´Tycoon´	1968
Harpswell Haze	´Blue Brilliant´ x unknown X ´Fourfold White´	1977
Harpswell Velvet	Hybrid between ´Blue Brilliant´, ´White Swirl´, ´Violet Flare´, ´Pirouette´, ´Polly Dodge´, ´Tealwood´	1990
Marcus Perry	unknown	1997
Navy Brass	´Orville Fay´ X ´White Swirl´ x ´Violet Flare´	1973
Orville Fay	´Violet Flare´ x unknown X ´Pirouette´ x unknown	1969
Pansy Purple	´Pirouette´ X unknown	1969
Sea Shadows	´White Swirl´ X ´Tycoon´	1964
Supernatural	unknown	1994
Wiltrud Giessel	unknown	1978
Zweiters Hundred	´Breiter Start´ X SSTT101	1984

The colours of the flowers were measured with the RHS colour chart, 5th edition from year 2007 with 203 pages (812 colours). Colours were measured under the conditions of full sun light and we measured the most common (covering the largest area) colour in falls and standards of 10 flowers. The colour that was most common was then determined as the colour of standards and falls. Results from this measurement are in the Tab 2.

Then we take from each taxon 80 grams of standards and falls. They were lyophilized, homogenized and used for the preparation of the extract. Extraction was conducted in the following manner: 3 grams of the dry sample were put into the methanol, acidified by the hydrochloric acid (1:50, Penta, Czech Republic) and homogenized for 5 minutes (VORTEX Genius 3, IKA, Deutschland). The obtained homogenized mass was centrifuged (10 min, 16.000 g, Eppendorf 5430R, Czech Republic). For the measurement itself was used optimized HPLC with DAD detector, detection goes on by λ=520 nm. Retention time of the measured samples together with the spectra was compared to the spectra and retention times of the six most common anthocyanin in the flowers (cyanidin-3- galaktoside, cyanidin-3-glukoside,

peonidin-3-glukoside, pelargonidin-3-glukoside, malvidin-3-glukoside, and delphinidin-3-glukoside). According to the wave length 520 nm, which is specific for anthocyanin we tried to detected these colorants. Although we measured several peaks for each examined sample, the positive reaction for the concrete six anthocyanin colours was weak. The only sample with a positive reaction was *I. sibirica* ´Elfelde´ in which we surly prove presence of delphinidin-3-glykozide and maybe of malvidin-3-glykozide. All the results from this measurement are in Tab 4.

For the dyeing process we used common water soaking. Pieces of 100% cotton fabrics were soaked in bleach and one sample in bleach and vinegar (8%). Dyeing was performed at 22 ± 1 °C for different time periods (24 hours, 48 hours and 72 hours) using a fixed amount (50g or 100g) of Iris perianth leaves. Dyed samples were extensively washed with cold and hot water to remove any unfixed dyed material and finally dried at an ambient temperature. After drying the colour of the dyed cloth was measured by the RHS colour chart. The results from this measurement are in Tab 5.

Today, the most commonly used instruments for measuring colour are spectrophotometers. Spectro-technology measures reflected or transmitted light at many points on the visual spectrum, which results in a curve. Since the curve of each colour is as unique as a signature or fingerprint, the curve is an excellent tool for identifying, specifying and matching colour. We used a Konica Minolta Spectrophotometer to measure the exact colours of the dyed cloth samples. This machine works with the reflected light and is primary used to check the maturity of fruits, vegetables or to identify the colours of flowers of Azalea hybrids. Output of this measuring was colour defined on a so-called LAB colour scale based on the opposing-colours theory of colour vision, which states that two colours cannot be both green and red at the same time, nor blue and yellow at the same time. As a result, single values can be used to describe the red/green, the yellow/blue and lightness attributes. Measured results can be seen in Tab 6. in which L* defines results for lightness, a* denotes the red/green value and b* the yellow/blue value.

After six months the cloth samples were measured again by RHS colour chart. During this time the clothes were stored in a dark, dry environment at room temperature. Results from this measurement are in Tab 5.

Several tests were performed on the mixed flowers of *Iris sibirica* to confirm the presence of the chosen chemical compounds. The plant material, flowers, was dried at room temperature (22–24 °C). The dried material was homogenized on mechanical mill IKA MF10 basic (sieve 2 mm, speed 500 rpm). The homogenized dry material was soaked by 75% methanol, and left unattended for 24 hours. The solution was filtered and stored in fridge by -4°C. This basic solution was then used in all the tests.

For the testing, we used so-called "drop tests". Their benefits are that just a small amount of used solution and chemicals are used, the facility for quick evaluation, the use of common chemicals, easy preparation and the use of the same solution for all tests. A disadvantage is inaccurate assessment; one never knows the exact content of the compound. Qualitative determination of phytochemical components was carried out as per the standard procedure from (Ganthra et al., 2012, Divya Lekshmi, 2013). The tested compounds were:

Flavonoids – 1ml of extract with a few drops of dilute sodium hydroxide (NaOH) added. An intense yellow colour was produced in the plant extract which becomes colourless with the addition of few drops of dilute acid, indicating the presence of flavonoid.

Phenols – 1ml of extract and 2 ml of distilled water were added followed by few drops of 10% ferric chloride ($FeCl_3$). Appearance of blue or green colour indicates presence of phenols.

Qinones – 1ml of extract and 1ml of concentrated sulphuric acid (H_2SO_4) was added. Formation of red colour shows the presence of quinones.

Tannins – 2ml 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 – 2ml of extract, 20 ml of distilled water was added and shaken vigorously at warm conditions. The formation of honey comb like foam indicates the presence of saponins.

Cardiac glycosides – 5ml of extract was treated with 2 ml of glacial acetic acid containing a drop of ferric chloride ($FeCl_3$) solution. Afterwards it was underplayed with 1 ml concentrated sulphuric acid (H_2SO_4). A brown ring of the interface indicates a de-oxy sugar characteristic of cardenolites.

Terpenoids – 5ml of each extract was mixed with 2 ml of chloroform. 3ml of concentrated sulphuric acid (H_2SO_4) was then added to form a layer. A reddish brown precipitate colouration at the interface formed indicated the presence of terpenoids.

Alkaloids – 3ml of the extract, 3 ml of 1 % HCl was added with continuous stirring in a 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.

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

Results from all this measurements are in Tab 7.

Results

In the following tables are summarized results from all measurements as described in the material and method section.

Table 2. Main standards and falls colour

Cultivar name	Main falls colour (RHS code)	Main standards colour (RHS code)
Ann Dasch	93 C Violet-blue group	94 A Violet-blue group
Cambridge	91 A Violet-blue group	93 B Violet-blue group
Dark Desire	N 89 D Violet-blue group	N 89 A Violet-blue group
Dreaming Spires	N 89 C Violet-blue group	N 89 A Violet-blue group
Elfelde	N 89 A Violet-blue group	N 89 A Violet-blue group
Friendly Welcome	91 A Violet-blue group	N 89 A Violet-blue group
Grand Junction	N 88 B Violet group	N 89 D Violet-blue group
Harpswell Haze	N 89 D Violet-blue group	N 89 C Violet-blue group
Harpswell Velvet	90 A Violet-blue group	90 A Violet-blue group
Marcus Perry	92 A Violet-blue group	92 B Violet-blue group
Navy Brass	92 A Violet-blue group	N 89 D Violet-blue group
Orville Fay	91 A Violet-blue group	92 A Violet-blue group
Pansy Purple	N 87 Violet group	N 87 Violet group
Sea Shadows	91 A Violet-blue group	94 B Violet-blue group
Supernatural	N 81 A Purple-violet group	N 79 B Purple group
Wiltrud Giessel	N 88 B Violet group	N 89 B Violet-blue group
Zweiters Hundred	93 C Violet-blue group	N 89 C Violet-blue group

Table two contains the list of the main falls and standards colours measured by the RHS colour chart. All the used *Iris sibirica* cultivars have flowers in shades of violet or violet-blue colour, only ´Supernatural´ have purple flowers.

Table 3. Retent time and peak area for the used anthocyanins

Sample	Retent time [minutes]	Peak area
cyanidin-3-glykozide	9,596	13053,2
peonidin-3-glykozide	10,287	81,8
pelargonidin -3-glykozide	10,443	14058,6
malvidin-3-glykozide	11,455	618,4
delfinidin-3-glykozide	12,332	122,5
cyanidin-3-galaktozide	14,01	274,4

Table 4. Results for the HPLC measurement

Sample	Retent time [minutes]	Peak area
Ann Dasch	11,721	318,7
Ann Dasch	12,148	1508,3
Ann Dasch	13,103	1154,8
Cambridge	11,725	194,3
Cambridge	12,167	728,5
Cambridge	13,12	241,3
Dark Desire	11,061	1350,7
Dark Desire	11,68	636,3
Dark Desire	12,106	2393,4
Dark Desire	12,767	368,7
Dark Desire	13,076	901,9
Dreaming Spires	11,126	412,1
Dreaming Spires	11,743	740
Dreaming Spires	12,181	2216,6
Dreaming Spires	12,834	182,3
Dreaming Spires	13,149	437,2
Elfelde	**11,49**	**127,6**
Elfelde	11,918	1109,3
Elfelde	**12,334**	**3712**
Elfelde	12,999	286,5
Elfelde	13,286	794,1
Friendly Welcome	11,276	704,8
Friendly Welcome	11,899	131,1
Friendly Welcome	12,308	828,3
Grand Junction	11,725	223,9
Grand Junction	12,164	1011,4
Grand Junction	13,123	285,9
Harpswell Haze	11,714	453,5
Harpswell Haze	12,154	1658,9
Harpswell Velvet	11,708	413,1
Harpswell Velvet	12,151	1510,3
Marcus Perry	11,873	122,7
Marcus Perry	12,298	537,4
Navy Brass	11,719	252,7
Navy Brass	12,165	971,6
Navy Brass	13,115	674
Orville Fay	11,638	288,9
Orville Fay	12,059	1098,6
Orville Fay	12,984	336
Pansy Purple	11,244	823,6
Pansy Purple	11,879	462,4
Pansy Purple	12,287	1742,7
Pansy Purple	13,234	850,1
Sea Shadows	11,277	525,4
Sea Shadows	11,914	364,8
Sea Shadows	12,328	1131,9
Sea Shadows	13,268	446,5
Supernatural	11,119	534,3
Supernatural	11,721	1324,8
Supernatural	12,157	3522,6
Supernatural	12,806	580,7
Supernatural	13,114	1415,4
Wiltrud Gissel	11,083	292,1
Wiltrud Gissel	11,705	1261,7
Wiltrud Gissel	12,135	3810,7
Wiltrud Gissel	12,799	506,5

Wiltrud Gissel	13,092	1165
Zweiters Hundred	11,245	617,9
Zweiters Hundred	11,869	869,6
Zweiters Hundred	12,288	2328,8
Zweiters Hundred	12,957	214
Zweiters Hundred	13,24	495,1

In tables three and four are the outputs from the HPLC DAD measurements. In table three are the six used anthocyanins ordered by the retention time. In table four are all the used *Iris sibirica* cultivars, ordered alphabetically and by the retention time. Two samples that have some similarity in retention time to the anthocyanins are highlighted in bold.

Table 5. Results from the measuring dyed cloths by RHS colour chart

Sample	RHS color chart number (freshly dyed)	RHS color chart number (after 6 months)
Soaking 24 h / boiled	N 88 C	97 C
Soaking 24 h / vinegar	92 C	N 170 C
Soaking 24 h	92 C	92 D
Soaking 48 h	108 A	97 C
Soaking 72 h	92 A	N 170 D
Soaking 72 h / 100g	92 B	N 170 D

Table 6. Results from measuring dyed cloths by Konica Minolta Spectrophotometer

Sample	Lightness (L)	Red/green (a)	Yellow/blue (b)
Soaking 24 h / boiled	80	1	-9
Soaking 24 h / vinegar	74	4	-7
Soaking 24 h	76	3	-7
Soaking 48 h	82	1	-8
Soaking 72 h	79	3	-7
Soaking 72 h / 100g	83	3	-5

In tables five and six are the results from the measuring of the freshly dyed cloths. In table five by the RHS colour chart and in table six by the Konica Minolta spectrophotometer.

Table 7. Results from drop tests

Tested group of chemicals	Result
Alkaloids	- Weak reaction
Phenols	++ very strong reaction
Flavonoids	- - No reaction
Quinones	++ very strong reaction
Saponins	- Weak reaction
Cardiac glycosides	++ very strong reaction
Glycosides	- Weak reaction
Tannins	- Weak reaction
Terpenoids	++ very strong reaction

In table seven are summarized the results from drop tests. For the valuation of the reaction strength we use a five point scale in which ++ was the strongest reaction, + was some reaction, 0 was inconclusive result, - was weak reaction and - - was no reaction at all.

Discussion

Yabuya et al. (1994b) strongly suggested that the bluing effect on flower colour of bluish purple cultivars of *Iris ensata* Thunb. was dependent on the co-pigmentation of anthocyanins with flavones. Asen et al. (1970) mentioned that flavone C-glycosides act as co-pigments in blue flowers of several species, including *Iris*. If the blue colour is transportable from flowers to cloths is not clear, but water soluble flavonoids, responsible for the red and blue colours of flowers and fruits, can be used as watercolours (Melo, 2008). We did not

attempt to examine the content of anthocyanins and flavones, but we have weak reaction for flavonoids.

Tannins are chemicals whose name was introduced by Seguin in 1790 to describe plant extractions that can convert raw hide to leather (Haslam, 1966). They have the propensity to give a greenish or bluish-black hue in the presence of iron salts (Cardon, 2007). At times they serve the dual purpose of mordant and colorant due to their close association with some of the other colouring groups such as flavonoids and quinones (Haslam, 1966). In our experiment the tannins have just a weak reaction so they will not be the chemicals responsible for the cloth dyeing.

Anthocyanins are important for successful attainment of flower colour breeding. Research of Yabuta et al. (2000), proved presence of 16 types of major anthocyanins in *Iris ensata* Thunb. Among these types, delphinidin 3RGac5G was useful for the breeding of blue flower colour and cyanidin 3RGac5G and peonidin 3RGac5G for the breeding of magenta flower colour.

The most famous colorant from *Iridaceae* is *Crocus sativus* L. which gives a rich yellow-golden colour (Attokaran, 2011). But there are also some Iris species which are mentioned in literature as a source of dye colours. For example the rhizomes of *Iris pseudacorus* L. are mentioned as source of black colour for cloth dyeing. The final colour was called as „Sabbath black“ due to sulphur used as stabilizer (Komarnicki, 1993, Allen and Hatfield, 2004). Ozturk et al. (2013) among the dye plants of Turkey with medicinal uses mentioned *Iris germanica* L., *Iris paradoxa* Steven and *Iris iberica* Hoffm. ssp, *elegantisima* (Sosn.)Takht. & Fedorov whose flowers are used as dyes and medicinal plants.

Historically there are four pigments which were made from irises (Eaustaugh et al., 2008). First is Catasol **(**green pigment).Nunes (1615) mentioned preparation of this colour from crushed iris parts, most likely from leaves. Green pigment achieved from iris leaves is also mentioned by Komarnicki (1993).

The second pigment is Iris Blue (Blue pigment). Due to different descriptions in three medieval manuscripts we can assume that this pigment was made from iris flowers after the pollen was taken from them (Eaustaugh et al., 2008).

The third is Iris Green (Green pigment) which was made from perianth leaves of various plants. The pigment was prepared by simply squeezing the juice from the flowers and mixing with an aluminium hydroxide (alum) base (Eaustaugh et al., 2008). Schweppe (1993) lists several plants that can be used to obtain this pigment, but the best is *Iris germanica* L. *Iris germanica* L. is mentioned as a source of green colour also by Dogan et al., (2002). Thompson (1956) in his book also mentioned irises as source for green colour. The green colour was made from the perianth leaves of the purple iris. The dye was initially purple, adding alum will mordant it to blue, and adding calcium will turn it green.

The last one is Mangiferin (Yellow pigment) sometimes called as iris yellow. The generic compound the xanthone mangiferin, 2--D-glucosidyl-1,3,6,7-tetrahydroxy- 9H-xanthen-9-one, is the principal colouring matter derived from the leaves of *Iris germanica* L. (Schweppe, 1993).

Better colour strength results are dependent on the metal salt used (Kamel et al., 2009), because we did not use any metal salts as fixatives the colour degradation was very quick.

Iris flowers meet several requirements for the natural dyes, which are, according to Bechtold (2003): Reasonable requirements for production and harvesting of the plant materials, easy handling and storage of the raw materials, easy extraction with water, simple and rapid dyeing process, no intermediate drying steps, etc., one-bath dyeing, bio-degradability of dyes in waste water treatment plants, non-toxic properties of dyes and non-allergic potential of dyed material, consumption of chemicals and energy comparable or

lower than the current state-of-the-art systems based upon synthetic dyestuffs (Bechtold et al., 2003).

Conclusion

Results of our research show that the plants of *Iris sibirica* L. have good dyeing potential. Although our results were not promising in the cotton cloth test, the results from all the other experiment show that the chemical possibilities and basics for the next research are present. Usage of different mordant will make the colors more sustainable and will lead to other promising results. So this study opens wide range of possibilities for the next experiments and testing in the future.

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