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TOTAL FLAVONOIDS AND PHENOLICS CONTENT OF THE CHOSEN GENUS *IRIS* SPECIES

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Abstract

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This article contains the results of the measurements of the flavonoids and phenolics content in the five *Iris* species (*Iris pseudacorus, Iris crocea, Iris spuria, Iris orientalis* and *Iris ensata)*. Chosen plants are mostly grown as ornamental plants because of their colourful flowers, but biochemical research in recent year show that these species also contain in their leaves, roots and flowers some interesting chemical substances that can be used in medicine. In this experiment were used 5 years old plants which were grown on the experimental grounds of Horticulture Faculty in Lednice. For the research were used rhizomes, because the rhizomes are by most authors considered as the richest source of the secondary metabolites. We used lyophilized rhizomes – to measure the content of flavonoids and phenolics in the fresh matter. And air dried rhizomes, which were dried in room temperature (22 °C) for fifteen and fifty days. The results show that the content of the flavonoids in the fresh (lyophylized) rhizomes was highest in the rhizomes of *Iris pseudacorus* and lowest in the rootstocks of *Iris orientalis*. The highest phenolics content in the fresh (lyophylized) rhizomes was also in the *Iris pseudacorus*, the lowest in the *Iris crocea*.

Iris pseudacorus, Iris crocea, Iris spuria, Iris orientalis, Iris ensata, flavonoids, phenolics

Phenolics probably constitute the largest group of plant secondary metabolites. Widespread in nature, and found in most classes of natural compounds having aromatic moieties, they range from simple structures with one aromatic ring to highly complex polymeric substances (ATTA-UR-RAHMAN, 2008). Phenolics are important constituents of some medicinal plants and they are used in the food industry as colouring agents, flavourings, aromatizers and antioxidants. Flavonoids are natural polyphenolic substances widely distributed in the different parts of plants such as fruits, bark, stems, roots, leaves and flowers. Structurally they are characterized by a pyran ring or a similar structure of three carbons. These polyphenolic compounds are well known for displaying a remarkable spectrum of biological activities, including antibacterial and antifungal properties (ATTA-UR-RAHMAN, 2008). In essential oils from different plants from the Iridaceae have been isolated more than thirty different iridals, unusual phytogenic triterpenoids with mono-, bi- or tricyclic structures essential oils. But the only derivatives of the iridals was their fatty acid esters at C-3.1 Only iridal glycosides were found in extracts of I. spuria (Zeal), cultivated in Egypt (MARNER *et al.*, 2002). The researches of Wink (2010), show that the neoflavonoids, which represent an alternative mode of cyclization of the phenylpropene and triketide precursor, were detected also in Iridaceae (*Iris*). A flavonoid survey of Iris species showed the characteristic constituents were glycoflavones but here they co-occur with isoflavones and the xanthone mangiferin and its derivatives. The rhizomes of *Iris tenuifolia* (*Iridaceae*) are the source of the largest number of new 2'-O-substituted simple flavanones in a single species. Additionally, the 2'-oxygenated dihydroflavonols corresponding to flavanones were found in the same species (ANDERSEN and MARKHAM, 2006). Phytochemical investigations on *Iris* species have resulted in the isolation of a variety of compounds including flavonoids, isoflavonoids and their glycosides, benzoquinones, triterpenoids and stilbene glycosides (KHAN and ATHER, 2006). According to Glasby (1991),

the most common chemicals in Irises are; ISOFLAVONES represented by iridin, irifloroside, irigenin, irilone, 4′,5,7-trihydroxy-3′,6-dimethoxyisoflavone, 3′,4′,5′-trihydroxy-6,7-dimethoxyisoflavone, 3′,4′,5′-trihydroxy-6,7-methylenedioxyisoflavone, irigenin, irisflorentin, junipigenin A, iristectorigenin, iristectorin A, tectoridin, NORSESQUITERPENOIDS represented by irone, β-irone, τ-irone, XANTHONES represented by irisxanthone, isomangiferin, isoswertiajaponin, isoswertisin, mangiferin, swertisin, PHENOLICS represented by acetovanillone, irigenin, irisolidone, STEROID represented by β-sitosterol, tocopherol, TRITERPENOIDS represented by α-amyrin, β-amyrin, iridogermanal, α-iridogermanal, τ-iridogermanal, δ-iridogermanal, ISOPRENOID QUINONES represented by 3-demethylplastoquinone 8, 3-demethylplastoquinone 9, phylloquinone, plastochromanol 8, plastoquinone 9, TERPENOIDS represented by iriflorental, iripallidal, AMINOACIDS represented by τ-glutamyl-β-alanine, τ-glutamyl-β-aminoisobutyric acid, FLAVONES represented by irigenin, iristectorigenin A, kanzakiflavone 1, kanzakiflavone 2, irisolidone. The compounds isolated from *Iris* species were reported to have piscicidal, antineoplastic, antioxidant, antitumor, antiplasmodial and antituberculosis properties. Andrews, as well as some isoflavonoids obtained from the rhizomes of *Iris* L. species have a noticeable anti-inflammatory activity when compared with (KHAN and ATHER, 2006). Iris flowers also contain some interesting chemical compounds. For example flowers of the wild forms of Iris ensata contain 6 types of anthocyanins, malvidin 3-(p-cumaroyl)-rutinosido-5-glucoside (malvidin3RGac5G)-petunidin 3-(p-cumaroyl)-rutinosido-5-glucoside (petunidin 3RGac5G), petunidin 3RGac5G - malvidin 3RGac5G, malvidin 3RGac5G, petunidin 3RGac5G, delphinidin 3-(p-cumaroyl)-rutinosido-5-glucoside(delphinidin 3RGac5G) and malvidin 3-rutinosido-5-glucoside(malvidin 3RG5G) – petunidin 3-rutinosido-5-glucoside(petunidin 3RG5G). Among these types, delphinidin 3RGac5G type is noteworthy because it is useful for the breeding of blue flower color. But further analysis of anthocyanins by high-performance liquid chromatography (HPLC) procedures show that also cyanidin 3-(p-cumaroyl)-rutinosido-5-glucoside (cyanidin 3RGac5G) and peonidin 3-(p-cumaroyl)-rutinosido-5-glucoside (peonidin 3RGac5G), useful for the breeding of red and magenta flowers can be found in flowers of this specie (YABUYA *et al.*, 1994). In the flowers of *Iris ensata* were also found some interesting chemicall compounds. For example, anthocyanin 5-O-glucosyltransferase, containing malvidin and petunidin 3-(pcoumaroyl) rhamnosylglucoside-5-glucosides, as well as nonacylated 3-rhamnosyl-glucoside-5-glucoside of these anthocyanidins as major anthocyanins (YAUBIA *et al.*, 2002).

Iris pseudacorus Linnaeus, 1753

Rhizomes are pink, freely branching, producing extensive clumps, 2–3 cm in diameter, with fibrous remains of old leaves; roots fleshy. Stems usually 1-branched, solid, 70–150 cm. Leaves are basal, deciduous, at first erect, then recurved, blade dark green, with prominent median thickening, 40–100 cm x 2–3 cm, slightly glaucous basally; cauline equaling inflorescence unit. Inflorescence units 4–12-flowered; spathes green with brown margins, outer spathe strongly keeled, inner without keel, 6–9 cm, subequal, margins not scarious. Flowers are perianth bright yellow; floral tube 0.6–0.8 cm, with no constriction into ovary; sepals bright yellow or cream colored, lanceolate to ovate or suborbiculate, 5–7.5 x 3–4 cm, signal a darker yellow basal patch limited by short, brown lines; petals without veining, lanceolate to spatulate, 2–3 cm; ovary triangular in cross section with concave sides and narrow groove at each angle, 1.5 cm; style keeled, 3–4 cm, crests spreading, 1–1.2 cm, laciniate at apex; stigmas rounded with prominent tongue; pedicel 2.5–7 cm. Capsules prismatic to oblong-ovoid, obscurely 3-angled with obvious groove at each angle, 3.5–6 cm, beak 5 mm. Seeds D-shaped, flattened, 6–7 mm, corky, lustrous. 2n = 34. Native in swamps, wet shores of rivers, and lakes. Native in Eurasia introduced in North America and Africa.

Iris crocea Jacquem, 1936

Plant is up to 150 cm height. Leaves are 60–90 cm long x 1.5–2.5 cm wide, ensiform. There is terminal cluster of the flowers at the tip of the flower stem and 3 lateral clusters on short erect branches. Flowers are 12-18 cm in diameter, deep golden yellow. Blade of the falls 4.5–5.0 cm x 2–2.5 cm; oblong, tapered, crimpled at margin, narrowing to 3–3.5 cm long haft. Standards are 7.5 cm long, oblanceolate, waved at edges, style 3.8 cm; crest deltoid. Capsule is 3.8–4.0 cm long, oblong, 6-angled, beaked. This iris is native in Kashmir, in mountains to the height 1600–2000 m.

Iris spuria Linnaeus, 1753

Rootstock is somewhat slender, rhizomes are hard and remain clothed with the base of old leaves, which do not split readily into fibres. Leaves are upright, stiff, ribbed, dark green in colour, subglaucescent, linear-ensiformis, tapering gradually to an acute point, 0.8–1.2 cm by 30 cm at flowering time, but growing long subsequently. Stem is about 2.5–3 cm, round, sheathed in 3 or 4 reduced leaves, which entirely hide the internodes and bearing a terminal head of two flowers and sometimes one or two lateral spicate heads, each of a single flower. Spathe valves are firm green, somewhat inflated, lanceolate and remaining green long after the flowers are over, the outer valve alone being slightly keeled 1.2–3 cm. The base colour of falls has red purple veins on a white ground. On the blade these veins become a deep blue purple, and the ground-work

is of a slightly paler shade of the same colour. The central ridge is greenish yellow with fait purple dots. Standards are slightly shorter than the falls, oblanceolate-unguiculate, of a deep violet-blue colour, slightly edged with yellow in the lower part. Some authors characterize the range of colours of this kind from soft blue, purple-blue, blue-violet, and purple to grey-lilac and white with varying colours of veining. Capsule is oblong, beaked with a double ridge at each angle, 2.5–5 cm long. Seeds are brown, smooth, more or less cubical, enveloped in a loose, dark, papery covering. Europe, Middle East, central Asia. *Iris spuria* has many subspecies. It is a widely distributed species that can be found growing trough Europe into Scandinavia and down to Spain, from France to Russia, and trough Iran to Turkey and then across to China.

Iris orientalis Miller, 1768

Rhizomes are sparingly short-branched, forming dense clumps, 1–1.5 cm diameter, hard, with old leaf bases at nodes; roots fleshy. Stems slightly flattened, with 1–2 short branches, solid, 40–120 cm. Leaves are basal erect, blade with slight spiral twist and central ridge, 35–80 cm x 1–2 cm, stiff, harsh, fibrous, glaucous; cauline 2–3, 1–2 subtending floral clusters, blade reduced. Inflorescence units clustered, 2–4-flowered; spathes white, 3–5 cm, subequal, papery. Flowers are perianth white; floral tube funnelform, 1–2.5 cm; sepals spreading and arching downward, with large yellow basal area, broadly orbicular, 8–10 x 3–6 cm, apex rounded, deeply emarginate; petals white, spatulate, 4–6 x 1–1.5 cm, base gradually attenuate, apex emarginate; ovary triangular in cross section with 2 ribs at each angle, 2–2.5 cm; style white, with parallel sides, 4–5 cm, crests erect, triangular, 1–2 cm; stigmas 2-lobed; pedicel 2.5–7.5 cm. Capsules ovoid to oblong-elliptic, triangular in cross section, each angle 2-ribbed, 4–5 × 2–2.5 cm. Seeds in 2 rows per locule, white, flattened or wedge-shaped, 4–5 mm, papery, wrinkled. 2 n = 40. Persisting after cultivation or discarded along roadsides; distributed mainly in Greece, Turkey.

Iris ensata Thunberg, 1794

Rhizomes are creeping, thick. Leaves are linear, 30–80 cm x 0.5–1.2 cm, midvein distinct on both surfaces, apex acuminate. Flowering stems 25–100 cm, solid, 1–3-leaved; spathes 3, lanceolate, unequal, 4.5–7.5 × 0.8–1.2 cm, leathery, 2-flowered, veins distinct, raised, basal spathe shorter, apex usually acute, apical spathe longer, apex usually obtuse. Flowers dark reddish purple, 9–10 cm in diam.; pedicel 1.5–3.5 cm. Perianth tube 1.5–2 cm; outer segments obovate, mottled yellow at center, 7–8.5 x 3–3.5 cm; inner segments erect, narrowly lanceolate, 5 cm × 5–6 mm. Capsule ellipsoid, 4.5–5.5 × 1.5–1.8 cm, 6-ribbed, apex shortly beaked. Seeds are maroon-brown, semiorbicular, and flat. 2 n = 24. This iris specie is native in damp areas along rivers and near lakes; 400–1700 m in Japan, Korea, and Russia.

MATERIAL AND METHODS

Experimental ground is situated on the full sun with spring irrigation. Plants grow in rows oriented in north-south direction, with the spacing between plants 70 cm and with spacing between rows 100 cm. Soil type is loamy, Chernozem. Soil analysis from the 2008 show that the content of the whole nitrogen is 1510 mg.kg^{-1}, nitrogen in the form of NO_3^- ion was 84.5 mg.kg^{-1}. Value of pH was 7.7. Content of the phosphorus was 456 mg.kg^{-1}, content of potassium was 363 mg.kg^{-1}, content of calcium 6090 mg.kg^{-1} and content of magnesium 510 mg.kg^{-1}.

Each year were plants treated with the doze of fertilizer (granulated NPK fertilizer). The fertilizer was hand splayed in spring months in amount 3 kg of the fertilizer on the whole area of the experimental ground. The area was three times in year weeded by hand, and once a year was area between rows weeded by herbicide (Roundup).

According to the Köppens classification the experimental ground is in the Cb_b, area, or in the Cb_{bx} area with the climatic signs of the temperate zone as the equal distribution of the precipitations during the year, mild summer and mild warm winter. January temperature is higher than –3 °C and Julys temperature is lower than 22 °C. Subtype x represents higher amount of the precipitations in the end of spring and in the beginning of summer with the subsequent dryer period. By adding agroclimatical regionalization we can specify that the area is in the macro area warm, with the sum of active temperatures higher than 2800 °C, sub area mostly dry with the value of climatic irrigation between 100–150 mm, precinct with T_{min} above –18 °C (ROŽŇOVSKÝ and LITSCHMAN, 2011).

For the analysis were used the rhizomes of five chosen plants concretely the *Iris pseudacorus, Iris crocea, Iris spuria, Iris orientalis* and *Iris ensata*. All used irises belong to the *Limniris* group.

Method

From all used plants were obtained 50 g of fresh rhizomes. The rhizomes were washed, cleaned from substrate and peeled from the upper skin, and roots. 40 g of the cleaned rhizomes were cut to smaller pieces and dried in room temperature for 15 days (in tables and graph marked as 2. analysis) and 50 days (in tables and graph marked as 3. analysis). 10 g were chopped and put for 24 hour to the lyophylisator directly after digging and cleaning to dry them as fresh as possible (in tables and graph marked as 1. analysis). From the both lyophylized and air dried material were made the same analysis, the content of total flavonoid and phenolics.

For the both analysis was prepared extract, with which, the analysis itself was made. The dried rhizomes samples of each taxon were grind in universal grinding mill (maximum grain size 3 mm).

I: *Overview of the chemical compounds find in Iris species used in our experiment as are reported in literature*

Iris pseudacorus L., 1753	irisin, irisquinone A ($C_{24}H_{38}O_3$), glycoside, iridin, myristic acid, pectinic substances, hemicelluloses	JIAJU *et a*l., 2011; KHARE, 2007; SANAVOVA and RAKHIMOV, 2004;
Iris crocea Jacquem, 1936	12a-hydroxyrotenoid, 9-methoxyirispurinol, tectorigenin 4'-glucoside, tectoridin, tectorigenin, 5,7-dihydroxy-6,2'-dimethoxyisoflavone, alipinone	SHAWL and KUMAR, 1992
Iris spuria L., 1753	iridalglycoside 5a ($C_{48}H_{80}O_{21}$), iridalglycoside 5b ($C_{42}H_{70}O_{16}$), iridalglycoside 6a ($C_{48}H_{80}O_{21}$), iridalglycoside 6b ($C_{48}H_{80}O_{21}$), iridalglycoside 6c ($C_{42}H_{70}O_{16}$), iridalglycoside 7 ($C_{42}H_{72}O_{16}$), iridalglycoside 8 ($C_{42}H_{72}O_{16}$), tectorigenin 7-O-β-d-glucopyranoside-4′-O-[β-d-glucopyranosyl-(1´→6′)-β-d-glucopyranoside], iristectorigenin B 4′-O-[β-d-glucopyranosyl-(1′→6´)-β-d-glucopyranoside], tectorigenin 7-O-β-d-glucopyranoside-4′-O-β-d-glucopyranoside, tectorigenin 4′-O-[β-d-glucopyranosyl-(1′→ 6´)-β-d-glucopyranoside], tectorigenin 7-O-β-d-glucopyranoside, genistein 7-O-β-d-glucopyranoside, tectorigenin 4′-O-β-d-glucopyranoside, tectorigenin, vanillic acid 4-O-β-d-glucopyranoside, glucosyringic acid, E-coniferin, maesopsin 6-O-β-d-glucopyranoside, 4-(2-formyl-5-hydroxymethylpyrrol-1-yl) butyric acid, 12a-dehydrorotenoid 1	FARAG *et al*, 2009; JIAJU *et al*., 2011 a; JIAJU *et al*., 2011 b; JIAJU *et al*., 2011 c; JIAJU *et al*., 2011 d; JIAJU *et al*., 2011 e; SINGAB, 2004
Iris orientalis Miller, 1768		
Iris ensata Thunberg, 1794	xanthone glycosides, C-glycoside of apigenin, phenolic acids - ferulic acid ($C_{10}H_{10}O_4$), p-coumaric acid ($C_9H_8O_7$), vanillic acid ($C_8H_8O_4$), p-hydroxybenzoic acid ($C_7H_6O_3$), ceryl alcohol, 5-hydroxy-4'-methoxyflavone, 5-hydroxy-3'-methoxyflavone, 5-hydroxy-2'-methoxyflavone, luteolin 6-C-B-D-glucopyranoside, magniferin ($C_{19}H_{18}O_{11}$), isomagniferin ($C_{19}H_{18}O_{11}$), 5-hydroxy-4´-methoxyflavone, 5-hydroxy-3´-methoxyflavone, 5-hydroxy-2´-methoxyflavone, malvidin 3RGac5G, petunidin 3RGac5G, delphinidin 3RGac5G, isovitexin	KHARE, 2007; BOLTENKOV *et al*., 2005; PRYACHINA and BLINOVA, 1984; BLINOVA and KALYUPANOVA, 1974; PRYACHINA and BLINOVA, 1979; BOLTENKOV *et al*., 2005; YABUYA *et al*., 1997; YABUYA *et al*., 2000

Small amount of each dried and crushed rootstock (2 g) was added to 50 ml of the 75% methanol and kept at the room temperature in digestor for 24 hours. The prepared samples were occasionally mixed. The content of total phenolics in each methanol extracts was determined according to the Folin-Ciocalteu procedure as described by YOU *et al*. (2007). Diluted methanol extract (250 µl) was mixed with 9 ml of distilled water and 1 ml of Folin-Ciocalteu reagent and allowed to stand at room temperature for 5 min. Sodium bicarbonate solution (10 ml, 7%) was added to the mixture and incubated at room temperature for 90 min and the absorbance was measured at 765 nm, using spectrophotometer (JENWAY 6100). The total phenolic content was expressed as grams of Gallic Acid in 100 g of plant matter (GAE/100g). All samples were analysed duplicately and averaged. The content of total flavonoid in each methanol extracts was determined according to the Aluminium chloride spectrophotometric method described by Zloch *et al.* (2004). Diluted methanol extract (50 µl), prepared day before, was mixed with 1.5 ml of distilled water and 0.2 ml NaNO2 (5%) and kept in room temperature for 5 min. Aluminium chloride solution (0.2 ml, 10%) was added to the mixture, shaken vigorously and incubated for 5 min. And finally sodium hydroxide solvent (1.5 ml, 1 mM) and 1 ml of distilled water were added to mixture, shaken vigorously and incubated for 15 min. The absorbance was measured at 510 nm, using spectrophotometer (JENWAY 6100). The total flavonoid content was expressed as grams of Catechin in 100 grams of plant matter. All samples were analysed in duplicate and averaged.

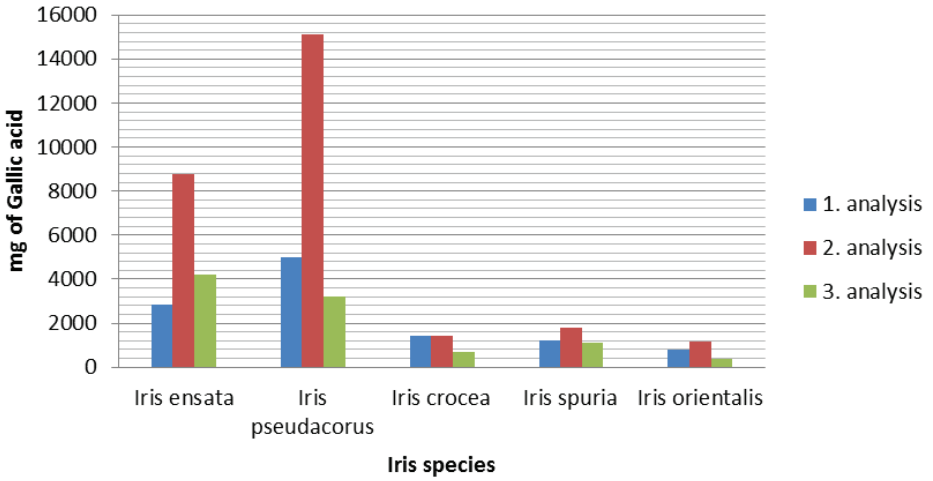
RESULTS

The measured values for the phenolics content, expressed in milligrams of the Gallic acid, are in the Tab. II.

From the results you can see that the highest phenolics content in the fresh matter (lyophilized rhizomes) was in the rhizomes of the *Iris pseudacorus* 5000.5 mg of the Gallic acid. The lowest content was in the rhizomes of *Iris orientalis* just 793.5 mg of the Gallic acid. The second measurement, done after 15 days of the drying in room temperature, show increasing amount of the Gallic acid in four samples (*I. ensata, I. pseudacorus, I. spuria, I. orientalis*). Just the sample of *I. pseudacorus* show three time higher levels of the phenol content compared with the sample made from fresh rhizomes. In the sample prepared from *Iris crocea,* the level of the phenol content was the same in the fresh matter and also in the samples

II: *Content of total phenolics expressed as milligrams of the Gallic acid in 100 g plant matter*

	Fresh matter [mg] (1. analysis)	Air dried 15 days [mg] 2. analysis)	Air dried 50 days [mg] (3. analysis)
Iris ensata	2 850	8 757	4 188
Iris pseudacorus	5 000.5	15 113	3 192
Iris crocea	1 450	1 453	714.44
Iris spuria	1 200.5	1 797	1 125.6
Iris orientalis	793.5	1 178	372.77



1: *Graphic display of the content of total phenolics expressed as milligrams of Gallic acid in 100g plant matter in three analysis*

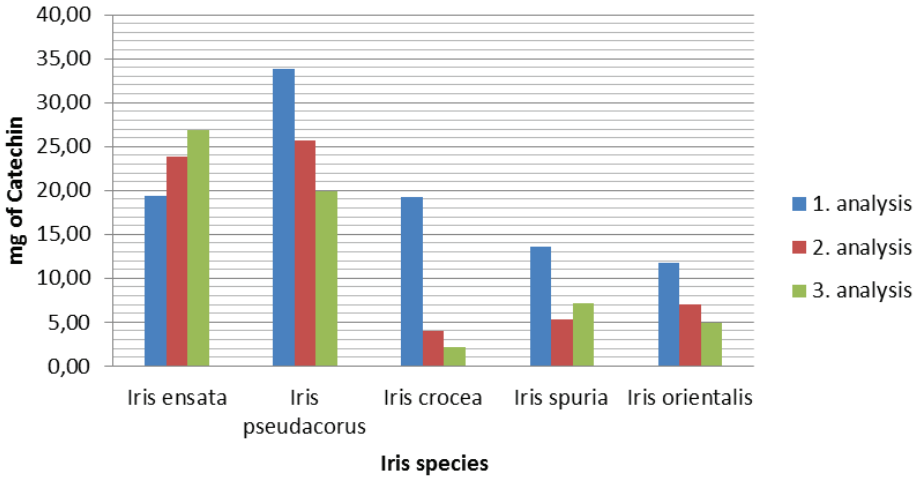
III: *Total flavonoid content expressed as milligrams of the Catechin in 100 g plant matter*

	Fresh matter [mg] (1. analysis)	Air dried 15 days [mg] (2. analysis)	Air dried 50 days [mg] (3. analysis)
Iris ensata	19.33	23.84	26.84
Iris pseudacorus	33.79	25.75	19.90
Iris crocea	19.31	3.,94	2.11
Iris spuria	13.61	5.32	7.09
Iris orientalis	11.76	7.06	4.90

after the 15 days of drying. The last measurements, done after 50 days of drying in room temperature, show significant decrease on the phenolics content, when compared with the fresh sample, in all species except the *Iris ensata* in which the amount of the GA was after 50 days twice as high as in the fresh matter. For the better display of the measured results for the total phenolics, are all the measured values expressed as mg of Gallic acid shown in the Fig. 1.

The measured values for the total flavonoid content, expressed in milligrams of the Catechin, in the 100 grams of the fresh plant matter are in the Tab. III.

From the results you can see that the highest flavonoid content in the fresh matter (lyophilized rhizomes) was in the rhizomes of the *Iris pseudacorus* 33 mg of the Catechin. The lowest content was in the rhizomes of *Iris orientalis* just 11.76 mg of the Catechin. The second measurement, done after 15 days of the air drying in room temperature, show decreasing amount of the Catechin content in four used species (*I. pseudacorus, I. crocea, I. spuria, I. orientalis*), just the sample of *I. ensata* show higher levels of the phenol content compared with the sample from fresh rhizomes. The last measurements done after 50 days of drying in room temperature, show decrease on the flavonoid content in four species *I. pseudacorus*, *I. crocea, I. spuria I. orientalis,* when compared with the fresh sample. The *Iris ensata*, show the increasing trend of the flavonoid content which was increasing with the time of drying. For the better display of the measured results of the total flavonoid content are all the measured values expressed as mg of Catechin shown in the Fig. 2.



2: *Graphic display of the total flavonoid content expressed as milligrams of the Catechin in 100 g plant matter*

DISCUSSION

The results from articles show that the different isoflavonoids and flavonoids were isolated from the plants which belong to genus *Iris* (WU and XU, 1992; MORITA *et al*., 1972). Williams *et al*. (1997) confirmed presence of flavonoids, xanthones and isoflavones in leaf, flower and rhizomes of several cultivars of bearded irises. Due to HARBORNE and WILLIAMS (1994) flavonoid aglycones have been found as major constituents on the leaf surface (external flavonoids) in *I. reichenbachii* and *I. pseudopumila*. Phytochemical investigations resulted in the isolation of several flavonoids (SHIBATA, 1927; MORITA *et al*., 1972 a; MORITA *et al*., 1972 b; XU *et al*., 1999; SHAN, 2007; YUAN *et al*., 2008). These articles confirmed our results which was relatively high content of total flavonoids and phenolics in the rootstocks of the used Iris plants.

Results from the several authors (JIAJU *et al*., 2011; KHARE, 2007; SANAVOVA and RAKHIMOV, 2004; SHAWL and KUMAR, 1992) confirm that in *Iris pseudacorus* have been found a large number of chemical compounds from which some belongs to the phenolic group. These results were confirmed also by our measurements in which we found the highest content of phenolics and flavonoid content in the *Iris pseudacorus* sample. The second biggest content both for phenolics and flavonoids was in the samples from *Iris ensata*. And also this species was confirmed in various researches as a rich source of different chemical compounds (KHARE, 2007; BOLTENKOV *et al*., 2005; PRYACHINA and BLINOVA, 1984; BLINOVA and KALYUPANOVA, 1974; PRYACHINA and BLINOVA, 1979; BOLTENKOV *et al*., 2005; YABUYA *et al*., 1997; YABUYA *et al*., 2000).

According to Schieber (2001), the loss of macromolecules like flavonoid during heat treatment might be due to the harsh drying conditions, in particular, the used temperature and duration. Similarly, DAVEY *et al*. (2000) reported that wet thermal processing can affect the phytochemicals by thermal breakdown that affect the integrity of the cell structure which then resulted in the migration of components, leading to losses by leakage or breakdown by various chemical reactions involving enzymes, light and oxygen. Various researchers have reported the degradation of phytochemicals upon thermal treatment. Zhang and Hamauzu (2004) discovered significant reductions of 72%, 66% and 65% of total phenolics, ascorbic acid and antioxidant activity in broccoli florets during thermal treatement. Study on the effect of the different drying methods on flavonoids degradation found that freeze drying treatment resulted in the lowest degradation as compared to that of vacuum drying and air drying (MOHD ZAINOL *et al*., 2009).

From these articles is clearly seen that the flavonoid content should decreased with the time and used method. In our case, the lyophilized rootstocks have higher content as the air dried rootstocks which confirmed the Mohd Zanol results from 2009, about the correlation between way of drying and flavonoid contend in samples. Also the results of the Schieber were confirmed, when with the longer time, the content in the air dried rootstocks was decreasing.

Auto-oxidation refers to the formation of cross-linked structures as a result of exposure to light and oxygen. Under the influence of light, oxygen can abstract a proton, thereby generating a radical. This is particularly likely to occur if the proton is adjacent to a double bond, because the radical electron can be delocalized, thus lowering the energy. Given their aromatic nature, phenolic compounds are easily autooxidized. The radical that is generated can subsequently react with other radicals to form a dimer. Since the radical electron is delocalized, several structures can be formed depending on the precise location of the radical electrons at the time of the reaction (WILFRED, 2006).

This was confirmed also by our research, when the content of the total phenolics in rootstocks was

decreasing with the passing time. This was caused by the relatively high air temperature and access of oxygen which have possibility to oxidize the phenolic compounds in rootstocks.

CONCLUSION

Our research proves that the *Iris pseudacorus, Iris crocea, Iris spuria, Iris orientalis* and *Iris ensata* are rich in the content of the flavonoids and phenolics. The highest content of phenolics, overall for the whole experiment, when expressed as miligrams of Gallic acid in 100 g of the plant matter was in the rootstocks of the *Iris pseudacorus* dried in the room temperature for 15 days. These results correspond with the phenol life cycle, when lot of phenolic compounds are made over time, in our case during the drying of the plant material. The lowest content of the phenolics was in rootstocks of the *Iris orientalis*, dried in the room temperature for the 50 days.

For the flavonoid content, when expressed as miligrams of the Catechin in 100 grams of the plant matter, the best results, highest content, was measured in the sample made from the rootstocks of *Iris pseudacorus.* Concretely it was in the sample that was just lyophilized, so we can say it was content in the fresh roots. The lowest content was in the rootstocks of *Iris crocea* rootstock which were dried in the room temperature for 50 days.

SUMMARY

To the research of the flavonoids and phenolics content in the plants from the genus Iris is devoted big number of researchers from different countries for many years. The advanced technologies in last decade help researchers not just to discover these chemical substances in the roots, rhizomes, flowers and green parts of the plants but also to determinate them and describe their chemical properties. But the research is mostly oriented just on the plants that already have some research history, were used in traditional medicine or ethnomedicine, or on species that are already used in medicinal, or in perfumery industry. Just in the last few years researchers try to use the presence of specific chemical compounds also as a tool for systematic botany to describe and determine the relations between species. The data from the field of the systematic botany can be then used as a start point for the search for the new sources of chemical compounds in the relatives of surveyed plants. Our experiment prove that the rootstocks of used iris species content a rather high amount of phenolics and flavonoids, which are the chemicals responsible for the medicinal properties of this genus, and so this plants can be used as source of the phenolic compounds and flavonoids for next research.

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