

Molecular and phytochemical characterization of F1 *Streptocarpus* hybrids and antioxidant potential of their flower extracts

Monica HÂRȚA^{1a}, Doru PAMFIL¹, Orsolya BORSAI^{2b}, Rodica POP³,
Doina CLAPA⁴, Zorița DIACONEASA⁵, Cristian R. SISEA^{3*}

¹University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Life Sciences Institute Regele Mihai I, 3-5 Mănăștur, 400372, Cluj-Napoca, Cluj, Romania; monica.harta@usamvcluj.ro; dpamfil@gmail.com

²AgroTransilvania Cluster, Dezmir, Apahida, Crișeni St. FN, Cluj, 407039, Romania; orsolya.borsai@usamvcluj.ro

³University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Horticulture, 3-5 Mănăștur, 400372, Cluj-Napoca, Cluj, Romania; prodica64@yahoo.com; cristian.sisea@usamvcluj.ro (*corresponding author)

⁴University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Institute of Advanced Horticulture Research of Transylvania, 3-5 Mănăștur, 400372, Cluj-Napoca, Cluj, Romania; doinaclapa@yahoo.com

⁵University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Food Science and Technology, 3-5 Mănăștur, 400372, Cluj-Napoca, Cluj, Romania; zorita.sconta@usamvcluj.ro

^{ab}These authors contributed equally to this work and should be considered co-first authors

Abstract

Streptocarpus x hybridus is a very popular houseplant with colourful flowers arousing thus the interest of plant breeders. Its potential value is even higher since different chemical compounds could be identified and used for food and pharmaceutical industries. Therefore, the objectives of this study were: to determine genetic relationships between twelve F1 *Streptocarpus* hybrids and their genitors; to determine the total phenolic and flavonoid content; to evaluate the antioxidant activity of the analysed individuals. Although 12 out of the 24 RAPD primers detected polymorphism among the individuals, the UPGMA dendrogram did not show a very clear grouping pattern for flower colour. In contrast, the phytochemical dendrogram shed more light on the dissimilarities among *Streptocarpus* flowers in terms of their polyphenolic content. The total phenolic and flavonoid content of the flower extracts determined by the Folin-Ciocalteu and aluminium chloride colorimetric methods ranged from 680.02 to 2360.57 mg of GAE/100 g FW and from 12.52 to 78.36 mg QE/100 g FW. The radical scavenging activity against ABTS ranged from 27.17 to 130.35 μ M Trolox/g FW. Due to its ornamental value, H12 was screened by HPLC-PDA-ESI-MS to identify and quantify the chemical compounds involved in flower colour. Among the six compounds that were identified and quantified (*i.e.* cyanidin-3-O-galactoside, cyanidin-3-O-xyloside, cyanidin-3-O-glucoside, cyanidin-3-O-arabinoside, peonidin-3-O-glucoside and apigenin-6-C-glucoside), isovitexin reached the highest concentration 4183 μ g rutin/100 g FW. These preliminary results, showing high total polyphenolic content and ABTS antioxidant activity, suggest that *Streptocarpus* flowers could be a valuable source of antioxidants.

Keywords: ABTS; antioxidants; anthocyanins; cape primrose; DNA; flower; isovitexin; RAPD

Introduction

Streptocarpus x hybridus V., commonly known as cape primrose, is belonging to the *Gesneriaceae* family, being a perennial species, which is extensively cultivated worldwide as ornamental potted plant due to their beautiful flowers (Chaudhury *et al.*, 2010).

In the last decades, hybridizations were employed to obtain modern commercial hybrids with new flower colours and shapes, in order to increase their attractiveness on the flower market (Dibley, 2018). Currently, *Streptocarpus* flowers can be found in red, salmon, magenta, blue, purple, pink and white colours, with different hues and interesting pigmentation patterns of the petals (Afkhami-Sarvestani *et al.*, 2012).

The ornamental value of *Streptocarpus* hybrids has been usually assessed using different morphological descriptors for flowers, such as flower size, shape and colour (Dibley, 2008), which were the main traits chosen by plant breeders in artificial selections. Breeding programs that combine morphological descriptors with molecular markers could enable a more precise and effective characterization of the hybrids (Hârța *et al.*, 2020). In many ornamental plants, several types of molecular markers, such as random amplified polymorphic DNA (RAPD), sequence-related amplified polymorphism (SRAP), inter simple sequence repeat (ISSR), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) and microsatellites (SSRs), have been used successfully to evaluate the genetic relationships between hybrids and their genitors (Abe *et al.*, 2002; Rout and Mohapatra, 2006; Conceição *et al.*, 2011; Röper *et al.*, 2015; Ramzan *et al.*, 2018, Aros *et al.*, 2019). Previous reports show that among the PCR-based molecular markers, RAPD has proven to be a reliable, low-cost technique for hybrid identification and genetic relationship assessment in new varieties of plants (Kobayashi *et al.*, 1996a; Yulita and Mansur, 2012; Pharmawati and Macfarlane, 2013; de Oliveira Belo *et al.*, 2018).

As mentioned above, obtaining new *Streptocarpus* varieties has been an important goal of plant breeders for many years, but now this topic is becoming far more important because different chemical constituents of plants are identified and used in food and pharmaceutical applications. In this context, flavonoids are the most studied bioactive compounds due to their high variability and stability (Crawford and Giannasi 1982; Rieseberg and Ellstrand, 1993; López-Caamal and Tovar-Sánchez, 2014). Furthermore, the most important pigments responsible for flower coloration are flavonoids, carotenoids and alkaloids (Ren *et al.*, 2017). Among flavonoids, anthocyanins occur as glycosides together with their aglycones (anthocyanidins), which are derived from 2-phenylbenzopyrilium cation (Bruneton, 1999) and are responsible for red, pink, mauve, purple and blue colours of most of the flowers (Piovan and Filippini, 2007). Various modifications can appear in the structure of anthocyanins and their specific colour depending on co-pigments, metal ions and pH (Tanaka *et al.*, 2008). Other flavonoids, such as flavones and flavonols, cover the range from ivory white to pale yellow colours of flowers (Becker *et al.*, 2011).

Anthocyanin pigments have been traditionally used as natural dyes with low to no toxicity level (Mlcek and Rop, 2011; Cortez *et al.*, 2017), providing a large spectrum of colours from orange and red to purple and blue hues, thus satisfying the consumers' demand for different food and non-food products' coloration (Kelley *et al.*, 2002; Vankar and Srivastava, 2010; Crișan *et al.*, 2018; Pires *et al.*, 2019). Moreover, the use of flavonoids as natural dyes, highly contributes to human health improvement through their antioxidant properties (Ginova *et al.*, 2013; Koike *et al.*, 2015; Loizzo *et al.*, 2016; Petrova *et al.*, 2016; Khoo *et al.*, 2017; Pires *et al.*, 2018). The increased oxidative stress has been identified as a major causative factor of several diseases, including neurodegenerative and cardiovascular disease (Navarro-Gonzales *et al.*, 2014; Li *et al.*, 2017). In this regard, the antioxidant potential of plants drew special attention because they can prevent or delay oxidation (Kasote *et al.*, 2015).

Several scientific reports have already been published on the evaluation of phytochemical compounds and their antioxidant activity, in many edible and wild flower extracts (Li *et al.*, 2009; Kaisoon *et al.*, 2011; Li *et al.*, 2014; Zheng *et al.*, 2018), but, to the best of our knowledge, no reports have been published regarding the antioxidant potential of flower extracts from *Streptocarpus* hybrid plants.

Therefore, the aims of this study were to establish the genetic relationships among twelve F1 *Streptocarpus* hybrids and their genitors by employing RAPD markers, to determine the polyphenolic compounds and to assess the antioxidant potential of crude flower extracts. We consider that the identification and quantification of the main chemical compounds determining flower colour represent valuable information that can successfully be used to complete the morphological and genetic characterization of *Streptocarpus* hybrids, which are of a great interest for future breeding programs.

Materials and Methods

Plant material

In the present study, molecular and phytochemical analyses were performed on 12 F1 *Streptocarpus* hybrids and their genitors (♀ P1 - 'Black Panther', ♂ P2 - 'Slumber Song' and ♂ P3 'Snow White'). The hybrids resulted from the following two crosses: P1 x P2 and P1 x P3. The plant material was obtained as described by Hârța *et al.* (2020). Based on the flower colour, which was considered to be an important indicator for the ornamental value, six F1 *Streptocarpus* hybrids from each cross were selected for RAPD and phytochemical analyses (Table 1). Meanwhile, the hybrids were propagated by leaf cuttings as well and grown in greenhouse conditions (Hârța *et al.*, 2020) to ensure the complete data set for genetic and phytochemical analyses.

Isolation of genomic DNA and RAPD analysis

Streptocarpus leaves were collected from each parent and F1 hybrid and genomic DNA was isolated using the protocol published by Lodhi *et al.* (1994) and improved by Pop *et al.* (2003) and Bodea *et al.* (2016). DNA purity and concentration were determined with a NanoDrop-1000 spectrophotometer. To perform RAPD analysis, the DNA samples were diluted to 50 ng/μl using distilled water. In total, 24 RAPD primers were screened for PCR amplification on the 15 *Streptocarpus* samples, of which 12 primers yielded clear and reproducible banding patterns with high levels of polymorphism. PCR amplification of the DNA samples and electrophoretic separation of amplified products were carried out according to the protocol described by Hârța *et al.* (2018). PCR amplifications and agarose gel electrophoretic migrations were repeated twice for each primer to ensure the reproducibility of results.


Chemicals and reagents

All of the solvents and chemicals used in this study were purchased from Sigma-Aldrich (Darmstadt, Germany). The anthocyanin standards cyanidin-3-O-glucoside chloride, pelargonidin-3-O-glucoside chloride, cyanidin-3-O-galactoside (purity 90%), cyanidin-3-O-arabioside (purity 97%), cyanidin-3-O-glucoside, (purity 95%) and cyanidin (purity 95%) were purchased from Polyphenols AS (Sandnes, Norway).

Flower extract preparation

Fresh flower petals were harvested from each *Streptocarpus* sample two weeks after the first bloom, on the 20th of June, 2014. Acidified methanol (MeOH + 0.03% HCl) was prepared before proceeding the extraction. Each sample was finely grounded in liquid nitrogen using a mortar and pestle. Subsequently, 0.5 g of the grounded sample was mixed with 15 ml solvent. The coloured mixture was centrifuged at 4600 rpm for 5 min and then the supernatant was collected. The extraction procedure was repeated until the samples turned colourless. Further, the collected extracts were evaporated at 40 °C at reduced pressure, dissolved in a known amount of acidified water, and filtered through 0.45 μm Millipore filter. The extractions were carried out at room temperature.

Table 1. Flower colour and Royal Horticultural Society Colour Chart codes (RHSCC) of genitors and F1 hybrids selected for RAPD and phytochemical analyses

Samples	Parents and F1 hybrids	Flower colour	RHSCC code
1	P1 "Black Panther" 	dark violet, blue violet	83B, N88A
2	P2 "Slumber Song" 	purple pink, yellow	N73A, 4A
3	P3 "Snow White" 	white, pale yellow	155B, 14B
4	H1' 	pink violet	N82B
5	H2' 	pink violet	N77B
6	H3' 	purple pink, blue violet	72A, N88A
7	H4' 	violet	N82D
8	H5' 	dark violet, violet	83B, 82B
9	H6' 	purple pink, yellow, white	N74C, 6A, 155B
10	H7'' 	blue violet	N88C
11	H8'' 	pink violet, white	N77B, 155A
12	H9'' 	pink violet	N89A
13	H10'' 	blue violet, dark violet	N88C, 83B
14	H11'' 	blue violet, violet	N88C, 82B
15	H12'' 	blue violet, dark pink violet	N88A, N77A

*hybrids obtained from P1xP2 cross; **hybrids obtained from P1xP3 cross

Determination of total phenolic content

The total phenolic content (TPC) of all extracts was determined by Folin-Ciocalteu spectrophotometric method described by Singleton *et al.* (1999). Briefly, each sample (25 μ l) was mixed with 1.8 ml distilled water, Folin-Ciocalteu reagent (120 μ l) and 340 μ l Na₂CO₃ (7.5% in water). The obtained mixture was incubated for 90 min in the dark and at room temperature; then the absorbance of the samples was read at 750 nm using a BioTek microplate reader. The results were expressed as mg of gallic acid equivalents (GAE) per 100 g of fresh weight (FW).

Determination of total flavonoid content

The total flavonoid content (TFC) of the samples was determined according to the aluminium chloride colorimetric method as described by Zhishen *et al.* (1999). The analysed samples were diluted with sterile distilled water to a final volume of 5 ml and then 5% NaNO₂ (300 μ l) was added. After 5 min the obtained mixture was completed with 10% AlCl₃ (300 μ l) and after 6 min 1N NaOH (2 ml) was also added to the mixture. The obtained solution was mixed well and the absorbance was measured at 510 nm using a spectrophotometer (JASCO V-630 series). Total flavonoid content was expressed as mg quercetin equivalents (QE)/100 g of fresh weight (FW).

2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation-decolorization assay

This assay is based on the capacity of an antioxidant to scavenge the ABTS radical cation (ABTS⁺) as compared to a standard antioxidant (Trolox). The antioxidant activity of analysed samples was determined according to the protocol described by Arnao *et al.* (2001) and adapted to 96 wells microplate. The blue-green ABTS⁺ solution was prepared from a stock solution of 7 mM ABTS and 2.45 mM potassium persulfate and kept at dark and room temperature for 12-16 h. In order to obtain the working solution from the stock solution the ABTS was diluted with EtOH until the solution had an absorbance value of 0.700 \pm 0.02 AU at 734 nm. After obtaining the work solution, 20 μ l Trolox at different concentrations and flowers extracts were mixed with 170 μ l ABTS⁺ solution and the absorbance was measured after 6 minutes of incubation in the dark at room temperature, using a microplate reader (BioTek Instruments). The results were expressed as μ M Trolox/g FW.

LC-ESI-MS identification and quantification of anthocyanins

The anthocyanins and flavones were identified and quantified in H12 flower extract. This hybrid presented the highest polyphenolic content and the most valuable flower characters from ornamental point of view as previously reported by Hârța *et al.* (2020). The ESI-MS (Electrospray ionization coupled with mass spectrometry) analysis was performed on an Agilent 1200 system that was equipped with a binary pump delivery system LC-20 AT (Prominence), a degasser DGU-20 A3 (Prominence) and a diode array SPD-M20 UV-VIS detector (DAD). The separation of the compounds was achieved on an Eclipse XDB C18 column (4 μ m, 4.6 \times 150 mm).

The mobile phase consisted of solvent A - bidistilled water and 0.1% acetic acid/acetonitrile (99/1) v/v and solvent B-acetonitrile and acetic acid 0.1%. The gradient elution system was programmed as following: 0-2 min, isocratic with 5% (v/v) eluent B; 2-18 min, linear gradient from 5% to 40% (v/v) eluent B; 18-20 min, linear gradient from 40% to 90% (v/v) eluent B; 20-24 min, isocratic on 90% (v/v) eluent B; 24-25 min, linear gradient from 90% to 5% (v/v) eluent B; 25-30 min, isocratic on 5% (v/v) eluent B. Flow rate was set to 0.5 ml/min and column temperature was maintained at 25 °C. The chromatogram was monitored at 520 nm. Identification of the compounds and peak assignments were done using their retention time, UV-VIS spectra comparing with standards and previously published literature. The samples were analysed also by HPLC-ESI-MS to confirm the credibility of the results. The mass spectrometric data were obtained using a single quadrupole 6110 mass spectrometer equipped with an ESI probe. The measurements were performed in the positive mode with an ion spray voltage of 3000 V, and a capillary temperature of 350 °C. Data were collected

in full scan mode within the range 280-1000 m/z. Quantification of anthocyanins glycosides was carried using a cyanidin-3-O-galactoside standard, and the various compounds were expressed as μg cyanidin-3-O-galactoside/100 g FW. For the quantification of flavone glycosides standard curve rutin were used and the results were expressed as $\mu\text{g}/100$ g FW.

Data analysis

RAPD gel images were analysed using TotalLab TL120 software. The bands of amplified fragments were scored as present (1) or absent (0) for each of the RAPD primers and transferred to a binary matrix using MS Excel. The data of this binary matrix (representing all recorded bands with the selected RAPD primers) was used to compute the genetic distance matrix based on Nei's 1972 coefficient (Nei, 1972) with SIMGEND computational module of NTSYS program version 2.1. (Rohlf, 2000). In order to assess the genetic relationships between F1 hybrids and their genitors, the SAHN module was choose to construct an UPGMA dendrogram based on the dissimilarity matrix. The COPH module of NTSYS pc. 2.1 was then used to create a cophenetic value matrix from the dendrogram and compared with the dissimilarity matrix using the MXCOMP computational module in NTSYS.

Data for total phenolic and flavonoid contents and antioxidant activity of the flower extracts were subjected to ANOVA analysis using SPSS 20.0 software. The experiments were carried out in a completely randomized design in three repetitions. The data were expressed as means \pm standard error. Differences among the treatments were further analysed using Duncan's multiple range test at the significance level of $p \leq 0.05$.

Spearman's-Rho correlation coefficients were also calculated to check the relationships between antioxidant activity and total phenolic and flavonoid content (SPSS 20.0 software). For cluster analysis, the mean values of total phenolic and flavonoid contents recorded from each *Streptocarpus* flower extract were used to create the dissimilarity matrix (in SIMINT computational module of NTSYS pc. 2.1) based on Euclidean distances, and an UPGMA dendrogram was constructed in SAHN module of NTSYS 2.1 (Rohlf, 2000).

Results

Molecular characterization based on RAPD analysis

Twelve out of the 24 screened primers were able to amplify all *Streptocarpus* DNA samples and produce polymorphic and clear bands. The selected primers and the number of polymorphic bands revealed by each primer are presented in Table 2. The results show that the number of polymorphic bands ranged from 7 to 15 with an average of 9.3 bands/primer. The highest number of polymorphic bands (15) was generated by OPD 20 primer, whereas the lowest number of amplified polymorphic bands (7) was revealed by OPB 07 primer. Polymorphism percentage was then calculated which ranged from 46.6% (OPB 07) to 93.7% (OPD 20) with a mean percentage of 70.8%.

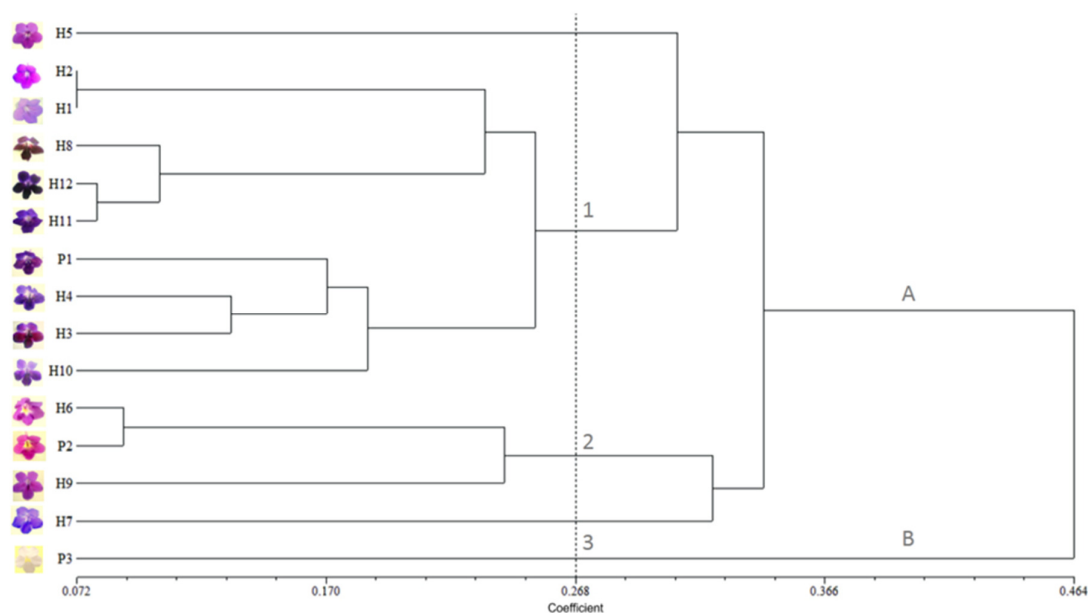
RAPD genetic relationships between genitors and F1 hybrids

In order to estimate the genetic relationships between genitors and F1 hybrids, cluster analysis was performed based on RAPD data. The value of cophenetic correlation coefficient between Nei's genetic distance matrix and the dendrogram matrix was $r = 0.948$, suggesting a very high goodness of fit (Rohlf, 2000).

The constructed UPGMA dendrogram successfully separated the analysed samples based on RAPD data as follows: two main clusters (A and B), leaving though "Parent 3" as an outlier. Within the first cluster (A) two subclusters were evidenced (1 and 2). Into the first subcluster nine hybrids were grouped together with P1 (\varnothing parent) while the second subcluster consisted of P2 and H6, H7 and H9 hybrids as shown in Figure 1.

Table 2. RAPD primers, total number of bands and polymorphism level (%)

No.	Primer	Sequence (5'-3')	Number of bands		Polymorphism (%)
			Total	Polymorphic	
1	OPA 03	AGTCAGCCAC	14	8	57.1
2	OPA 11	CAATCGCCGT	13	8	61.5
3	OPB 08	GTCCACACGG	15	11	73.3
4	OPB 07	GGTGACGCAG	15	7	46.6
5	OPB 18	CCACAGCAGT	14	9	64.3
6	OPAB 11	GTGCGCAATG	13	8	61.5
7	OPAL 20	AGGAGTCGGA	14	10	71.4
8	OPG 07	GAACCTGCGG	9	8	88.8
9	OPD 20	ACCCGGTCAC	16	15	93.7
10	OPF 02	GAGGATCCCT	13	10	76.9
11	OPH 02	TCGGACGTGA	11	8	72.7
12	OPI 03	CAGAAGCCCA	11	9	81.8
Average no. of bands/primer			13.2	9.3	70.8

**Figure 1.** UPGMA dendrogram of *Streptocarpus* genitors and F1 hybrids for RAPD data*Phytochemical characterization - total phenolic and flavonoid content*

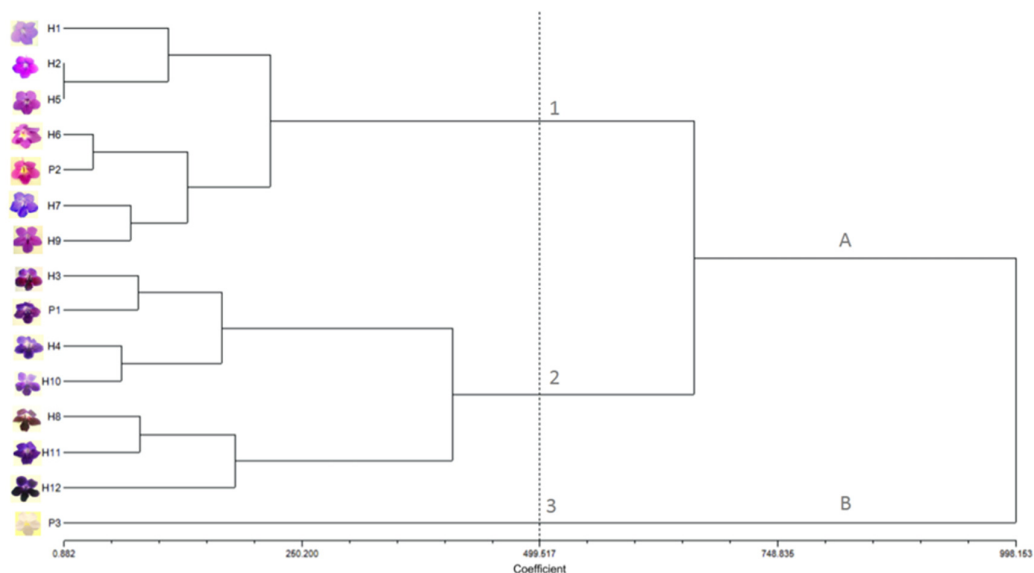
Fifteen *Streptocarpus* fresh flower extracts were analysed for their total phenolic and flavonoid content. The TPC values recorded in *Streptocarpus* extracts ranged from 680.02 to 2360.57 mg of GAE/100 g FW. Among the analysed samples, H12 had the highest total phenolic content (2360.57 mg of GAE/100 g FW), whereas P3 had the lowest (680.02 mg GAE/100 g FW). The results showed that H1, H2, H5 and H6 hybrids, resulted from the P1 x P2 cross, had a lower phenolic content than any of the parents, while H3 and H4 progenies of the same cross showed intermediate values in terms of phenolic content as compared to their parents. Statistically significant differences among the means of total phenolic content of the flower extracts are presented in Table 3. The total flavonoid content followed a similar variation pattern in the flower extracts as TPC; H12 had the highest TFC (78.36 ± 0.31 mg QE/100 g FW), while in P2 the lowest TFC was registered (12.52 ± 0.03 mg QE/100 g FW).

Table 3. Total phenolics (TP) flavonoids (TF) and antioxidant activity (ABTS) of flowers extracts

Sample no.	Genitors/ Hybrids	TP mg GAE/100 g FW)	TF (mg QE/100 g FW)	ABTS μ M Trolox/ g FW
1	P1	1950.44 \pm 2.40 ^{ghi}	40.56 \pm 0.36 ^{bc}	58.63 \pm 0.21 ^{bc}
2	P2	1390.08 \pm 0.88 ^{bc}	29.36 \pm 0.28 ^{ab}	49.6 \pm 0.15 ^b
3	P3	680.02 \pm 0.88 ^a	12.52 \pm 0.03 ^a	27.17 \pm 0.0 ^a
4	H1	1150.46 \pm 3.84 ^b	14.23 \pm 0.17 ^a	27.38 \pm 0.1 ^a
5	H2	1260.15 \pm 1.45 ^b	19.65 \pm 0.49 ^{ab}	46.31 \pm 0.25 ^{ab}
6	H3	1880.24 \pm 0.58 ^{efgh}	75.23 \pm 0.14 ^d	94.77 \pm 0.03 ^{de}
7	H4	1780.76 \pm 0.33 ^{defg}	58.46 \pm 0.11 ^{cd}	63.2 \pm 0.09 ^{bc}
8	H5	1260.09 \pm 1.86 ^b	20.53 \pm 0.33 ^{ab}	77.23 \pm 0.06 ^{cd}
9	H6	1360.79 \pm 1.76 ^{bc}	19.98 \pm 0.00 ^{ab}	46.32 \pm 0.25 ^{ab}
10	H7	1470.32 \pm 0.88 ^{bcd}	27.55 \pm 0.02 ^{ab}	44.6 \pm 0.06 ^{ab}
11	H8	2220.13 \pm 2.08 ^{hi}	75.81 \pm 0.01 ^d	89.32 \pm 0.23 ^d
12	H9	1540.89 \pm 1.20 ^{bcde}	33.63 \pm 0.05 ^{ab}	66.3 \pm 0.27 ^{bc}
13	H10	1720.45 \pm 2.33 ^{cdef}	63.69 \pm 0.30 ^{cd}	78.5 \pm 0.03 ^{cd}
14	H11	2140.38 \pm 1.00 ^{ghi}	70.81 \pm 0.09 ^d	110.25 \pm 0.13 ^{ef}
15	H12	2360.57 \pm 1.53 ⁱ	78.36 \pm 0.31 ^d	130.55 \pm 0.06 ^f

*Data shown are mean \pm standard error. Different lowercase letters indicate significant differences between the means of the same chemical compound at a significant level of 0.05 according to Duncan's test.

Based on the values of the chemical analyses (phenolics and flavonoids) an UPGMA dendrogram was constructed to estimate the relationships among three *Streptocarpus* genitors and twelve F1 hybrids (Figure 2). Figure 1 shows the identification of two main clusters (A and B) including all individuals, but P3 (outlier). Within the main cluster A, two subclusters were evident, each of them with two subgroups. The first subcluster consisted of P2 (σ parent) and H1, H2, H3, H5 and H6 hybrids resulted from the P1 x P2 cross and H7 and H9 resultants of the P1 x P3 cross. Regarding the second subcluster, P1 (ρ parent) was grouped together with H3, H4, H10, H8, H11 and H12, suggesting very similar flower colours and hues in their flowers.

**Figure 2.** UPGMA dendrogram of *Streptocarpus* genitors and F1 hybrids for phytochemical data

2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation-decolorization assay

The antioxidant activity of the analysed samples was found to be ranging between 27.17 and 130.35 μM Trolox/g FW with the highest antioxidant values recorded in H12 ($130.55 \pm 0.06 \mu\text{M}$ Trolox/g FW) and H11 ($110.25 \pm 0.13 \mu\text{M}$ Trolox/g FW). Statistically significant differences in terms of antioxidant activity were recorded in H3 and H5 and H8, H10, H11 and H12 as compared to their genitors from the first and second cross, respectively. The lowest antioxidant activity ($27.17 \mu\text{M}$ Trolox/g FW) was measured in P3 (Table 3). The results of Spearman's correlation show a strong positive relationship between the total flavonoid content and antioxidant potential ($r = 0.95$, $p = 0.001$) and also between total phenolic content and antioxidant potential ($r = 0.86$, $p = 0.001$) of the analysed samples.

LC-ESI-MS identification and quantification of anthocyanins

HPLC-PDA-MS identification of anthocyanins and flavone glucoside was made based on their retention time, UV-VIS spectra and mass spectral analysis compared with standards and literature data. The HPLC chromatogram for H12 flower extract with detection at 520 nm wavelength is presented in Figure 3.

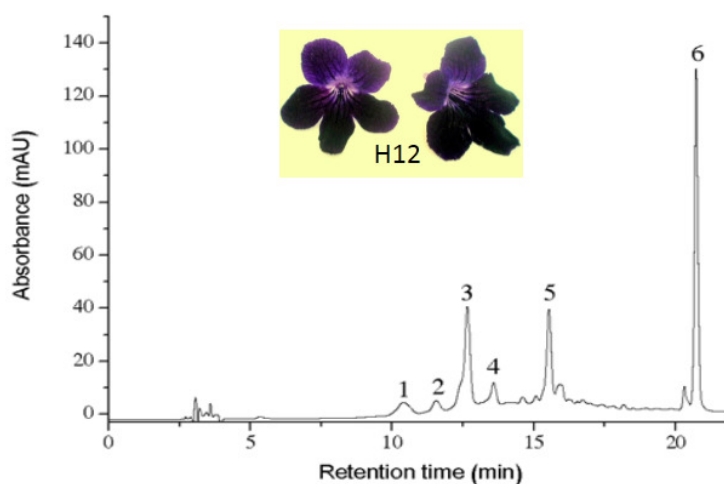


Figure 3. HPLC-PDA chromatogram of H12 sample recorded at 520 nm

According to their HPLC retention times (R_t), UV-VIS maximum absorption wavelengths and m/z values, six chemical compounds were identified in H12 flower extract, including five anthocyanins and one flavone glucoside. The concentrations of each identified compound are shown in Table 4.

Table 4. Retention times, UV-VIS max. absorption wavelengths, tentative identification, and concentration of anthocyanins and flavone glucoside in *Streptocarpus* H12 sample

Peak	R_t (min)	[M- H] ⁺ (m/z)	UV-VIS λ_{max} (nm)	Area (mAU)	Tentatively identified compounds	Concentration ($\mu\text{g}/100 \text{ g FW}$)
1	10.4	449, 287	525, 280	129	Cyanidin-3- <i>O</i> -galactoside	266*
2	11.5	419, 287	530, 282	74	Cyanidin-3- <i>O</i> -xyloside	165*
3	12.6	449, 287	525, 280	650	Cyanidin-3- <i>O</i> -glucoside	1221*
4	13.5	419, 287	530, 282	148	Cyanidin-3- <i>O</i> -arabinoside	301*
5	15.5	463, 301	530, 272	451	Peonidin-3- <i>O</i> -glucoside	856*
6	20.7	434, 272	340, 270	1093	Apigenin-6- <i>C</i> -glucoside	4183**

* expressed as μg Cy-3-*O*-gal/100 g FW; ** expressed as μg rutin/100 g FW

Discussion

RAPD markers, total phenolic and flavonoid contents and antioxidant activity have been used and determined in this study to evaluate the genetic variability among *Streptocarpus* hybrids and their genitors. Regarding RAPD molecular analysis, our results showed that the OPD20 primer identified a higher level of polymorphism (93.7%) compared to that reported for hybrids of *Rosa sinensis* (63.3%), by Prasad (2014), but lower than in *Grevillea* hybrids, where the polymorphism generated by OPD20 primer was 100 % (Pharmawati and Macfarlane, 2013). In this study, the lowest number of RAPD polymorphic bands (7) was generated by OPB 07 primer. This number was higher than that recorded by Van Staden and Pan (2001) in a study regarding the genetic diversity of blue-flowered *Scilla* species when OPB 07 primer generated 5 polymorphic bands in an artificial hybrid resulted from the cross between *Scilla natalensis* x *S. kraussii*.

To evaluate the RAPD genetic relationships between *Streptocarpus* parents and F1 progenies, cluster analysis was used. Although 12 out of the RAPD tested primers were able to detect polymorphism among individuals, it was not possible to establish a very clear grouping (link) among *Streptocarpus* genitors and their F1 hybrids based on the dissimilarities of the flower colour. It is worth mentioning that most of the F1 hybrids' flower colours were very similar to their mother genitor. Oehlkers (1964), in his report, claims that, in *Streptocarpus*, many characters such as flower colour have maternal inheritance being controlled by chromosomal genes and specific cytoplasmic components. Our results are in accordance with those reported by Rezende *et al.* (2009), who analysed the genetic divergence among some gerbera genotypes using RAPD markers, and concluded that flower colour traits were controlled by several genes located on different chromosomes and these genes may not be among those amplified by the tested RAPD primers. In some cases, depending on the species, few genes may be responsible for a single morphological character, such as flower colour (Rodrigues *et al.*, 2012). The earliest studies in *Streptocarpus* (Lawrence *et al.*, 1939) identified six genes (named: *V*, *F*, *R*, *O*, *D* and *I*) which were involved in the inheritance of seven flower colours: blue, mauve, magenta, rose, pink, salmon and white. As biochemical data became available, specific functions were assigned to these genes, such as the control of anthocyanidins and anthoxanthins patterning of petals (Lawrence and Sturgess, 1957).

In our study, the UPGMA dendrogram for phytochemical data shows that, based on their flower colours F1 hybrids were grouped with P1 and P2 genitors. Parent P3 has the white colour of flowers due to the possible recessive status of the complementary genes *V* and *F* (*vv ff*). These genes are both "dominant" and are required in the inheritance of anthocyanidins in flowers (Lawrence and Sturgess, 1957). However, Forkmann and Stotz (1984) in one of their report about *Streptocarpus* stated that the flowers lacked any colour because the hydroxylation of flavanones in the 3-position to dihydroflavonols was blocked.

Interesting is the fact that in this study none of the F1 *Streptocarpus* hybrids presented white colour of flowers. A possible explanation for this phenomenon could be that 'Snow White' (P3) used for the second parental cross is a diploid X-ray irradiated mutant from 'Maassen's White' - a mutation of the 'Constant Nymph' which has violet-blue flowers (Hârța *et al.*, 2020). Moreover, according to Lawrence and Sturgess (1957) the evolution of white flower colour seemed to be controlled by the mutation of successive genes becoming thus dominant and finally epistatic to their predecessors.

Flower colour is one of the major ornamental traits of *Streptocarpus*, but only a few studies have been carried out regarding the chemical profile of the flowers and how their colours are determined (Lawrence *et al.*, 1939; Lawrence and Sturgess, 1957; Lawrence, 1957; Forkmann and Stotz, 1984). In addition, the information about total polyphenols content and antioxidant capacity of *Streptocarpus* flower extracts are scant, however phenolic compounds are considered as a major group of compounds that contribute to the antioxidant activity of different parts of the plant. Our results show the total phenolic content of the *Streptocarpus* flowers' petal extracts ranging from 680.02 to 2360.57 mg of GAE/100 g FW which suggests that these flowers could be a very good source of antioxidants. These antioxidant levels are comparable to those reported by Garzón and Wrolstad (2009) for *Tropaeolum majus* orange petals (406 mg GAE/100 g FW) and *Rosa* × *hybrida* flower

petals (2087.43 mg GAE/100 g FW) as reported by Ge and Ma (2013). As expected, our experiment shows that light colour hybrids had smaller phenolic content whereas dark colour hybrids revealed higher phenolic content in the petals.

In this study, ABTS assay was also selected to measure the antioxidant potential of flower extracts. This colorimetric method can be applied for samples containing hydrophilic, lipophilic, and highly pigmented antioxidant compounds (Floegel *et al.*, 2011). In addition, ABTS assay represents a simple, fast, and cost-effective analysis of the antioxidant capacity (Diaconeasa *et al.*, 2019). In our experiment, the antioxidant potential of the analysed samples was found to range between 27.17 and 130.35 μM Trolox/g FW. In order to confirm the hypothesis that *Streptocarpus* flowers play an important role as antioxidants we compared the ABTS radical scavenging activity of the *Streptocarpus* petals, to some other species which have been recommended for food products due to their high antioxidant activity. With regard to the ABTS radical scavenging activity, *Streptocarpus* flowers presented superior values comparing with the ones reported by Borowska *et al.* (2009) for *Vaccinium oxycoccus* (16.4 μM Trolox/g FW), and very similar to the ones recorded by Garzón *et al.* (2010) and Sariburun *et al.* (2010) for *Vaccinium meridionale* (45.5 μM Trolox/g FW) and *Rubus idaeus* 'Hollanda Borum' (117.07 μM Trolox/g FW). Higher values of ABTS radical scavenging activity were recorded in *Rubus fruticosus* 'Jumbo' (146.89 μM Trolox/g FW) as reported by Sariburun *et al.* (2010).

According to Lawrence (1957), the main flavonoids involved in *Streptocarpus* flower colour are anthocyanidins. The anthocyanins are derivatives of delphinidin, cyanidin and pelargonin and exist also in the form of glycosides: 3-monoside, 3-pentoseglycoside and 3-5 dimonoside. Anthoxanthins (flavones, flavonols and flavanones) represent another group of sap-pigments and some of them have the property of making some anthocyanins bluer and these are described as co-pigments (Lawrence and Sturgess, 1957).

In this study, we identified and quantified six chemical compounds in flower extract of H12 sample as previously mentioned in Table 4. Our results are in agreement with those reported by Lawrence and Sturgess (1957) who claim that cyanidin monosides are the most common pigments associated with flowering plants.

An important finding of this study is that by HPLC analysis the presence of apigenin-6-C-glucoside in the flower extract was confirmed. In the earliest study reported by Lawrence and Sturgess (1957), it was presumed that anthoxanthins play the role of copigments, and these are probably apigenin or chlorogenic acid. In our study, we identified peonidin-3-O-glucoside which was described as a derivative (by partial methylation) of cyanidin. Especially in petals, flavone and flavonol glucosides are frequently found together with their in structure corresponding anthocyanins and conferring bright colours to *Streptocarpus* flowers (Bonner and Varner, 1965).

Apigenin-6-C-glucoside known as isovitexin was also identified in the flower extracts of *Crataegus pentagyna* (Prinz *et al.*, 2007) and in leaves' extracts of several ornamental plants such as *Ficus deltoidea* (Farsi *et al.*, 2013), *Mimosa pudica* (Zhang *et al.*, 2011), *Passiflora incarnata* and *P. caerulea* (Pereira *et al.*, 2005). Since recent studies show that flavonoids exhibit multiple pharmacological activities (Kumar and Pandey, 2013), isovitexin has gained increasing attention and importance due to its anti-cancer, anti-oxidant, anti-inflammatory, anti-nociceptive anti-Alzheimer's disease activities (He *et al.*, 2016). However, there is little information regarding the side effects and metabolic processes, since most of the current research reports are still pre-clinical (He *et al.*, 2016). Therefore, further data collection would be needed to understand better its metabolism and functions.

Conclusions

In summary, to the best of our knowledge this is the first report providing an evidence of genetic diversity based on RAPD molecular markers, antioxidant activities and polyphenol content of *Streptocarpus* hybrids and their genitors. These results widen the knowledge about different anthocyanins and anthoxanthins present in *Streptocarpus* flowers which has a decisive role in flower colour determination. However further

investigations are needed to explore and better understand the mechanisms underlying flower colour determination. We strongly believe that these preliminary results could serve as valuable information for future breeding activities on *Streptocarpus* × *hybridus*.

Authors' Contributions

Conceptualization (MH, ZD, CRS); Data curation (MH, ZD, OB, DC, RP); Formal analysis (MH, OB); Funding acquisition (OB, CRS); Investigation (RP, DC); Methodology (MH, ZD, RP, CRS); Project administration (MH, ZD); Resources (CRS); Software (MH; ZD; OB); Supervision (CRS, ZD); Validation (DC, DP); Visualization (OB, CRS); Writing - original draft (MH, OB, ZD, CRS); Writing - review and editing (MH, OB, ZD, CRS). All authors read and approved the final manuscript.

Acknowledgements

This work was supported by a grant of the Romanian Ministry of Education and Research, CCCDI - UEFISCDI, project number PN-III-P2-2.1-PTE-2019-0670 within PNCDI III and project 946/SMIS 14064: Institute of Advanced Horticulture Research of Transylvania (UASMVCN).

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Abe H, Nakano M, Nakatsuka A, Nakayama M, Koshioka M, Yamagishi M (2002). Genetic analysis of floral anthocyanin pigmentation traits in Asiatic hybrid lily using molecular linkage maps. *Theoretical and Applied Genetics* 105(8):1175-1182. <https://doi.org/10.1007/s00122-002-1053-7>
- Afkhami-Sarvestani R, Serek M, Winkelmann T (2012). Interspecific crosses within the *Streptocarpus* subgenus *Streptocarpella* and intergeneric crosses between *Streptocarpella* and *Saintpaulia ionantha* genotypes. *Scientia Horticulturae* (Amsterdam) 148:215-222. <https://doi.org/10.1016/j.scienta.2012.10.006>
- Arnao MB, Cano A, Alcolea JF, Acosta M (2001). Estimation of free radical-quenching activity of leaf pigment extracts. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques* 12(2):138-43. <https://doi.org/10.1002/pca.571>
- Aros D, Suazo M, Rivas C, Zapata P, Ubeda C, Bridge M (2019). Molecular and morphological characterization of new interspecific hybrids of alstroemeria originated from *A. caryophylleae* scented lines. *Euphytica* 215:93. <https://doi.org/10.1007/s10681-019-2415-4>
- Becker A, Alix K, Damerval C (2011). The evolution of flower development: current understanding and future challenges. *Annals of Botany* 107(9):1427-31. <https://doi.org/10.1093/aob/mcr122>
- Bodea M, Pamfil D, Pop R, Sisea RC (2016). DNA isolation from desiccated leaf material from plum tree (*Prunus domestica* L.) molecular analysis. *Bulletin UASVM CN (H)* 1:1-2. <http://dx.doi.org/10.15835/buasvmcn-hort:11761>
- Bonner J, Varner JE (1965). *Plant biochemistry*, Academic Press, San Diego
- Borowska EJ, Mazur B, Gadzała Kopciuch R, Buszewski B (2009). Polyphenol, anthocyanin and resveratrol mass fractions and antioxidant properties of cranberry cultivars. *Food Technology and Biotechnology* 47(1):56-61. <https://hrcak.srce.hr/33053>

- Bruneton J (1999). Anthocyanins. In: Pharmacognosy-phytochemistry medicinal plants (2nd edition). Intercepts, London.
- Chaudhury A, Power JB, Davey MR (2010). High frequency direct plant regeneration from leaf and petals of Cape Primrose (*Streptocarpus*). Journal of Crop Sciences and Biotechnology 13:1071-12. <https://doi.org/10.1007/s12892-010-0006-y>
- Conceição LDHCS, Belo GO, Souza MM, Santos SF, Cerqueira-Silva CBM, Corrêa RX (2011). Confirmation of cross-fertilization using molecular markers in ornamental passion flower hybrids. Genetics and Molecular Research 10(1):47-52. <https://doi.org/10.4238/vol10-Igmr894>
- Cortez R, Luna-Vital DA, Margulis D, Gonzalez de Mejia E (2017). Natural pigments: stabilization methods of anthocyanins for food applications. Comprehensive Reviews in Food Science and Food Safety 16(1):180-98. <https://doi.org/10.1111/1541-4337.12244>
- Crawford D, Giannasi D (1982). Plant chemosystematics. BioScience 32(2):114-124. <https://www.jstor.org/stable/1308564>
- Crișan I, Vidican R, Morea A, Simea Ș (2018). Dyeing potential of linen fabric with Iris flower extracts. ProEnvironment/ProMediu 11(36).
- Diaconasa Z, Iuhas CI, Ayvaz H, Rugină D, Stanilă A, Dulf F, ... Pinteș A (2019). Phytochemical characterization of commercial processed blueberry, blackberry, blackcurrant, cranberry, and raspberry and their antioxidant activity. Antioxidants 8(11):540. <https://doi.org/10.3390/antiox8110540>
- Dibley R (2008). *Streptocarpus*. Dibley Nurseries Handbooks. (2nd edition), Wales.
- Dibley R (2018). *Streptocarpus*. Dibley Nurseries Handbooks. (3rd edition), Wales.
- Farsi E, Shafaei A, Hor SY, Ahamed MB, Yam MF, Asmawi MZ, Ismail Z (2013). Genotoxicity and acute and subchronic toxicity studies of a standardized methanolic extract of *Ficus deltoidea* leaves. Clinics 68(6):865-75. [https://doi.org/10.6061/clinics/2013\(06\)23](https://doi.org/10.6061/clinics/2013(06)23)
- Floegel A, Kim DO, Chung SJ, Koo SI, Chun OK (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. Journal of Food Composition and Analysis 24(7):1043-8. <https://doi.org/10.1016/j.jfca.2011.01.008>
- Forkmann G, Stotz G (1984). Selection and characterisation of flavanone 3-hydroxylase mutants of *Dahlia*, *Streptocarpus*, *Verbena* and *Zinnia*. Planta 161(3):261-5. <https://doi.org/10.1007/BF00982923>
- Garzón GA, Narváez CE, Riedl KM, Schwartz SJ (2010). Chemical composition, anthocyanins, non-anthocyanin phenolics and antioxidant activity of wild bilberry (*Vaccinium meridionale* Swartz) from Colombia. Food Chemistry 122(4):980-986. <https://doi.org/10.1016/j.foodchem.2010.03.017>
- Garzón GA, Wrolstad RE (2009). Major anthocyanins and antioxidant activity of Nasturtium flowers (*Tropaeolum majus*). Food Chemistry 114(1):44-9. <https://doi.org/10.1016/j.foodchem.2008.09.013>
- Ge Q, Ma X (2013). Composition and antioxidant activity of anthocyanins isolated from Yunnan edible rose (An ning). Food Science and Human Wellness 2(2):68-74. <https://doi.org/10.1016/j.fshw.2013.04.001>
- Ginova A, Mihalev K, Kondakova V (2013). Antioxidant capacity of petals and leaves from different rose (*Rosa damascena* Mill.) plantations in Bulgaria. International Journal of Pure Applied Bioscience 1:38-43.
- Hârța M, Borsai O, Muntean CM, Dina NE, Fălămaș A, ... Ștefan R (2020). Assessment of genetic relationships between *Streptocarpus x hybridus* V. parents and F1 progenies using SRAP markers and FT-IR spectroscopy. Plants 9(2):160. <https://doi.org/10.3390/plants9020160>
- Hârța M, Clapa D, Borsai O, Rusu MC, Kelemen C, Pop R, Pamfil D (2018) Micropropagation and assessment of genetic stability of acclimatized *Streptocarpus x hybridus* Voss plantlets using RAPD markers. Bulletin UASVM. Horticulture 75:154-162. <http://dx.doi.org/10.15835/buasvmcn-hort:2018.0039>
- He M, Min JW, Kong WL, He XH, Li JX, Peng BW (2016). A review on the pharmacological effects of vitexin and isovitexin. Fitoterapia 115:74-85. <https://doi.org/10.1016/j.fitote.2016.09.011>
- Kaisoon O, Siriamornpun S, Weerapreeyakul N, Meeso N (2011). Phenolic compounds and antioxidant activities of edible flowers from Thailand. Journal of Functional Foods 3(2):88-99. <https://doi.org/10.1016/j.jff.2011.03.002>
- Kasote DM, Katyare SS, Hegde MV, Bae H (2015). Significance of antioxidant potential of plants and its relevance to therapeutic applications. International Journal of Biological Sciences 11(8):982. <https://doi.org/10.7150/ijbs.12096>
- Kelley KM, Behe BK, Biernbaum JA, Poff KL (2002). Combinations of colors and species of containerized edible flowers: Effect on consumer preferences. HortScience 37(1):218-21. <https://doi.org/10.21273/HORTSCI.37.1.218>

- Khoo HE, Azlan A, Tang ST, Lim SM (2017). Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food & Nutrition Research* 61(1):1361779. <https://doi.org/10.1080/16546628.2017.1361779>
- Kobayashi N, Akabane M, Handal T, Takayanagi K (1996a). Inheritance of morphological characters and RAPD markers in intersubgeneric hybrids of azalea, (*Rhododendron kiusianum* Makino × *R. indicum* (L.) Sweet) × *R. japonicum* (A. Gray) Suringer f. flavum Nakai. *Journal of the Japanese Society for Horticultural Science* 65(1):145-53.
- Koike A, Barreira JC, Barros L, Santos-Buelga C, Villavicencio AL, Ferreira IC (2015). Edible flowers of *Viola tricolor* L. as a new functional food: Antioxidant activity, individual phenolics and effects of gamma and electron-beam irradiation. *Food Chemistry* 179:6-14. <https://doi.org/10.1016/j.foodchem.2015.01.123>
- Kumar S, Pandey AK (2013). Chemistry and biological activities of flavonoids: an overview. *The Scientific World Journal* <https://doi.org/10.1155/2013/162750>
- Lawrence WJC (1957). Studies on *Streptocarpus*. IV. Genetics of flower colour patterns. *Heredity* 11:337-357.
- Lawrence WJC, Scott-Moncrieff R, Sturgess VC (1939). Studies on *Streptocarpus*. *Journal of Genetics* 38(1-2):299. <https://doi.org/10.1007/BF02982175>
- Lawrence WJC, Sturgess VC (1957). Studies on *Streptocarpus*. III. Genetics and chemistry of flower colour in the garden forms, species, and hybrids. *Heredity* 11:303-336.
- Li AN, Li S, Li HB, Xu DP, Xu XR, Chen F (2014). Total phenolic contents and antioxidant capacities of 51 edible and wild flowers. *Journal of Functional Foods* 6:319-30. <https://doi.org/10.1016/j.jff.2013.10.022>
- Li C, Du H, Wang L, Shu Q, Zheng Y, Xu Y, ... Ge Y (2009). Flavonoid composition and antioxidant activity of tree peony (*Paeonia section* Moutan) yellow flowers. *Journal of Agricultural and Food Chemistry* 57(18):8496-503. <https://doi.org/10.1021/jf902103b>
- Li D, Wang P, Luo Y, Zhao M, Chen F (2017). Health benefits of anthocyanins and molecular mechanisms: Update from recent decade. *Critical Reviews in Food Science and Nutrition* 57(8):1729-41. <https://doi.org/10.1080/10408398.2015.1030064>
- Lodhi MA, Guang-Ning Z, Weeden FNE, Reisch BI (1994). A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. *Plant Molecular Biology Reports* 12:6-13. <https://doi-org.am-e-information.ro/10.1007/BF02668658>
- Loizzo MR, Pugliese A, Bonesi M, Tenuta MC, Menichini F, ... Tundis R (2016). Edible flowers: a rich source of phytochemicals with antioxidant and hypoglycemic properties. *Journal of Agricultural and Food Chemistry* 64(12):2467-74. <https://doi.org/10.1021/acs.jafc.5b03092>
- López-Caamal A, Tovar-Sánchez E (2014). Genetic, morphological, and chemical patterns of plant hybridization. *Revista Chilena de Historia Natural* 87:16. <https://doi.org/10.1186/s40693-014-0016-0>
- Mlcek J, Rop O (2011). Fresh edible flowers of ornamental plants-A new source of nutraceutical foods. *Trends in Food Science & Technology* 22(10):561-9. <https://doi.org/10.1016/j.tifs.2011.04.006>
- Navarro-González I, González-Barrio R, García-Valverde V, Bautista-Ortín AB, Periago MJ (2014). Nutritional composition and antioxidant capacity in edible flowers: characterisation of phenolic compounds by HPLC-DAD-ESI/MSn. *International Journal of Molecular Sciences* 16(1):805-22. <https://doi.org/10.3390/ijms16010805>
- Nei, M (1972). Genetic distance between populations. *The American Naturalist* 106(949):283-292. <https://doi.org/10.1086/282771>
- Oehlkers F (1964). Cytoplasmic inheritance in the genus *Streptocarpus* Lindley. In: *Advances in Genetics*. Academic Press, New York.
- Oliveira de Belo G, Souza MM, Silva GS, Lavinsky MP (2018). Hybrids of Passiflora: *P. gardneri* versus *P. gibertii*, confirmation of paternity, morphological and cytogenetic characterization. *Euphytica* 214(1):2. <https://doi.org/10.1007/s10681-017-2021-2>
- Pereira CA, Yariwake JH, McCullagh M (2005). Distinction of the C-glycosylflavone isomer pairs orientin/isoorientin and vitexin/isovitexin using HPLC-MS exact mass measurement and in-source CID. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques* 16(5):295-301. <https://doi.org/10.1002/pca.820>
- Petrova I, Petkova N, Ivanov I (2016). Five edible flowers-valuable source of antioxidants in human nutrition. *International Journal of Pharmacognosy and Phytochemical Research* 8(4):604-10.

- Pharmawati M, Macfarlane IJ (2013). The genetic relationships of *Grevillea* hybrids determined by RAPD marker. HAYATI Journal of Biosciences 20(4):196-200. <https://doi.org/10.4308/hjb.20.4.196>
- Piovan A, Filippini R (2007). Anthocyanins in *Catharanthus roseus* *in vivo* and *in vitro*: a review. Phytochemistry Reviews 16(2-3):235-242. <https://doi.org/10.1007/s11101-006-9052-y>
- Pires TC, Dias MI, Barros L, Calhelha RC, Alves MJ, Oliveira MB, ... Ferreira IC (2018). Edible flowers as sources of phenolic compounds with bioactive potential. Food Research International 105:580-8. <https://doi.org/10.1016/j.foodres.2017.11.014>
- Pop R, Ardelean M, Pamfil D, Gaboreanu IM (2003). The efficiency of different DNA isolation and purification in ten cultivars of *Vitis vinifera*. Bulletin USAMV CN (ZB) 59:259-261.
- Prasad MP (2014). Molecular characterization and genetic diversity determination of *Hibiscus* species using RAPD molecular markers. Asian Journal of Plant Science and Research 4(3):50-56.
- Prinz S, Ringl A, Huefner A, Pemp E, Kopp B (2007). 4''-Acetylvitexin-2 ''-O-rhamnoside, isoorientin, orientin, and 8-methoxykaempferol-3-O-glucoside as markers for the differentiation of *Crataegus monogyna* and *Crataegus pentagyna* from *Crataegus laevigata* (Rosaceae). Chemistry & Biodiversity 4(12):2920-31. <https://doi.org/10.1002/cbdv.200790241>
- Ramzan F, Kim HT, Shim KK, Choi YH, Younis A, Lim KB (2018). Genetic diversity and relationship assessment of *Lilium lancifolium* x Asiatic hybrid 'Chianti' progeny by ISSR markers. European Journal of Horticultural Science 83(3):142-150. <https://doi.org/10.17660/eJHS.2018/83.3.3>
- Ren Y, Yang J, Lu B, Jiang Y, Chen H, Hong Y, ... Miao Y (2017). Structure of pigment metabolic pathways and their contributions to white tepal color formation of Chinese *Narcissus tazetta* var. *chinensis* cv Jinzhanyintai. International Journal of Molecular Sciences 18(9):1923. <https://doi.org/10.3390/ijms18091923>
- Rezende RK, Paiva LV, Paiva R, Chalfun Junior A, Torga PP, Masetto TE (2009). Divergência genética entre cultivares de gerbera utilizando marcadores RAPD [Analysis of genetic diversity between gerbera cultivars using RAPD markers]. Ciência Rural 39(8):2435-40. <https://doi.org/10.1590/S0103-84782009005000176>
- Rieseberg LH, Ellstrand NC (1993). What can molecular and morphological markers tell us about plant hybridization? Critical Reviews in Plant Sciences 12:213-241. <https://doi.org/10.1080/07352689309701902>
- Rodrigues MG, Mazzini RB, Pivetta KF, Alves MD, Desidério JA (2012). Characterization of the genetic variability among *Caesalpinia pulcherrima* (L.) Sw. (Fabaceae) plants using RAPD molecular markers. Acta Scientiarum Agronomy 34(3):259-63. <https://doi.org/10.1590/S1807-86212012000300005>
- Rohlf FJ (2000). NTSYS-pc: numerical taxonomy and multi-variate analysis system. Exeter Publ Setauket, New York.
- Röper AC, Orabi J, Lütken H, Christensen B, Skou AMT, Müller R (2015). Phenotypic and genotypic analysis of newly obtained interspecific hybrids in the *Campanula* genus. PloS ONE 10:e0137537. <https://doi.org/10.1371/journal.pone.0137537>
- Rout GR, Mohapatra A (2006). Use of molecular markers in ornamental plants: a critical reappraisal. European Journal of Horticultural Science 53-68. <http://www.jstor.org/stable/24126689>
- Sariburun E, Şahin S, Demir C, Türkben C, Uylaşer V. Phenolic content and antioxidant activity of raspberry and blackberry cultivars. Journal of Food Science 75(4):C328-35. <https://doi.org/10.1111/j.1750-3841.2010.01571.x>
- Singleton VL, Orthofer R, Lamuela-Raventós RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In Methods in Enzymology 299:152-178.
- Tanaka Y, Sasaki N, Ohmiya A (2008). Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. The Plant Journal 54 (4):733-749. <https://doi.org/10.1111/j.1365-313X.2008.03447.x>
- Van Staden J, Pan M (2001). Genetic diversity of blue-flowered *Scilla* species as determined by random amplified polymorphic DNA. South African Journal of Botany 67(2):344-348. [https://doi.org/10.1016/S0254-6299\(15\)31138-8](https://doi.org/10.1016/S0254-6299(15)31138-8)
- Vankar PS, Srivastava J (2010). Evaluation of anthocyanin content in red and blue flowers. International Journal of Food Engineering 6(4). <https://doi.org/10.2202/1556-3758.1907>
- Yulita KS, Mansur M (2012). The occurrence of hybrid in *Nepenthes hookeriana* Lindl. from Central Kalimantan can be detected by RAPD and ISSR markers. Hayati Journal of Biosciences 19:18-24. <http://dx.doi.org/10.4308/hjb.19.1.18>
- Zhang J, Yuan K, Zhou WL, Zhou J, Yang P (2011). Studies on the active components and antioxidant activities of the extracts of *Mimosa pudica* Linn. from southern China. Pharmacognosy Magazine 7(25):35. <https://dx.doi.org/10.4103%2F0973-1296.75899>

Zheng J, Yu X, Maninder M, Xu B (2018). Total phenolics and antioxidants profiles of commonly consumed edible flowers in China. *International Journal of Food Properties* 21(1):1524-40. <https://doi.org/10.1080/10942912.2018.1494195>

Zhishen J, Mengcheng T, Jianming W (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry* 64(4):555-9. [https://doi.org/10.1016/S0308-8146\(98\)00102-2](https://doi.org/10.1016/S0308-8146(98)00102-2)



The journal offers free, immediate, and unrestricted access to peer-reviewed research and scholarly work. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author.



License - Articles published in *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* are Open-Access, distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) License.

© Articles by the authors; UASVM, Cluj-Napoca, Romania. The journal allows the author(s) to hold the copyright/to retain publishing rights without restriction.