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Research Article

Separation and identification of individual anthocyanins from the petals of some rose cultivars

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Abstract

Rose cultivars represent a source with potential for therapeutical applications due to the high content in bioactive phytochemicals, including phenolic derivatives and lipophilic compounds, such as carotenoids. Anthocyanins, a phenolic subclass, represent the major compounds responsible for the colors of roses. In this study, we have aimed to extract, separate, and identify the anthocyanin compounds found in the petals of 25 different rose cultivars. High performance liquid chromatography followed by electrospray ionization mass spectrometry (HPLC-ESI-MS) was used to separate, identify and quantify the individual anthocyanins. The results showed the presence of 9 anthocyanin compounds, whereas the total anthocyanin content of rose cultivars ranged from 20.267-1206.841 mg/100 g FW, from which cyanidin-caffeoyl-glucoside was the most quantitatively represented anthocianin, followed by cyanidin-glucoside and pelargonidin-glucoside. In addition to this, the antioxidant activity was applied using the established DPPH *in vitro* method (1403 ± 301-304 ± 37 µmol TE/g FW), as well as the total phenolic content (2600±452-208±23 mg GAE/100 g FW) and the total flavonoid content (160±23-705±49 mg QE/100 g FW). Among the evaluated cultivars, the highest content in anthocyanin was determined for the 'Amalia' cultivar. Between the 25 rose cultivars analyzed, the varieties 'Amalia', 'Foc de tabără', 'Fruhrot', and 'First Red' followed as being the richest in anthocyanins content and the lowest values were recorded for the varieties 'Jimini Cricket', 'Cri-Cri', 'Fortuna', and 'Zburlici'. The same trend was observed for the total phenolic and total flavonoid content but also the antioxidant activity. The importance of analyzing anthocyanin content in different rose varieties came from the use of anthocyanins as natural dyes, having potential pharmaceutical ingredients that give various beneficial health effects.

Keywords: anthocyanins; antioxidants; HPLC; total phenolic content; total flavonoid content; rose cultivars

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Introduction

Anthocyanins are naturally occurring pigments that are responsible for the formation of varied colors, such as pink, red, violet, and blue. These secondary metabolites with polyphenolic backbone (belonging to the flavonoid class) are present in many plant species. They are located in all tissues, particularly in the intensely colored petals and fruits (Bueno *et al*., 2012). At cellular level, they are dissolved and evenly distributed in the acidic vacuolar juice or well-demarcated regions of the cell vacuole, after their synthesis takes place in the endoplasmic reticulum (Markham *et al*., 2000; Kitamura, 2006; Buhrman *et al*., 2022). From a chemical perspective, anthocyanins represent α- or β-glycosides of anthocyanidins (which are the aglycones), presenting a common central flavylium ion and several hydroxy and sometimes methoxy groups. The majority of anthocyanins are structurally anthocyanidin-3-*O*-glucosides (Kong *et al*., 2003), but glycosylation can also occur in the 5-, 7-, 3`-, or 5` positions. The glycosides may also be acylated with groups derived from phenolic (cinnamic, ferulic, or sinapic acid) or aliphatic acids (acetic, malic, succinic, oxalic, or malonic acid) *(Andrei et al*., 2014).

The genus *Rosa* of Rosaceae family contains more than 100 species that are naturally distributed in the flora of the northern hemisphere (Stănilă *et al*., 2015; Leus *et al*., 2018; Nicolescu *et al*., 2022). Rose (*Rosa* × *hybrid* L.) cultivars, belonging to this genus, are developed and used not only for their undisputable ornamental value, but also for their cosmetic, nutritional, and therapeutic benefits (Yang and Shin, 2017; Wan *et al*., 2019; Veluru *et al*., 2020). With more than 30,000 available cultivars, which have been developed through different hybridization programs to enhance desired ornamental properties (Salcă Roman *et al*., 2024), roses present a high potential for the assessment of phytochemical composition.

In rose petals, anthocyanins contribute to the vibrant red tones of the colors, but they can also be responsible for the rare blue and purple hues (Kumari *et al*., 2017). The most important derivatives are represented by cyanidin and pelargonidin glucosides, where *O*-glycosylation can occur in the positions 3 and/or 5 on the flavonoid backbone. Furthermore, anthocyanins derived from petunidin, delphinidin, malvidin, and peonidin have been identified before (Kumari *et al*., 2022; Ribeiro *et al*., 2024). Rose anthocyanins present a major potential in coloration applications, but they also stand out for their beneficial health effects, including their noteworthy antioxidant activity (Einbond *et al*., 2004; Salman *et a*l., 2023), as well as anti-inflammatory, antimicrobial, anti-tumoral, anti-aging, and hypoglycemic effects (Xue *et al*., 2022). From this point of view, rose petals present multiple prospective applications, based on their aesthetic appeal and bioactive potential. More critically, their color can also establish the commercial value of ornamental rose varieties (Schulz *et al*., 2016; Shahrin *et al*., 2015).

Considering the importance of rose anthocyanins the present study aimed to assess 25 less studied rose genotypes, through the determination of the individual anthocyanin compounds found in their petals, using a HPLC method coupled with UV-VIS detection. This step was applied to obtain information regarding the anthocyanin content of the genotypes, aiding future research efforts in this field. Moreover, complementary analyses have been applied using *in vitro* total phenolic and flavonoid content, as well as the DPPH antioxidant assay, with the aim of connecting the potential bioactive effects of the phenolic fractions in rose petals.

Materials and Methods

Rose cultivars

In this study, 25 rose varieties (native and from abroad) were studied during the summer of 2022 from the rose breeding laboratory and rose collection from the Horticultural Research Station (HRS), belonging to the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca. Roses meet favorable

ecological conditions in the study area, in the center of Transylvania, Romania (Salcă Roman *et al*., 2024). The collection of rose varieties was established in 1962 (200 varieties) and completed in 2000. Today it covers an area of 1.0 ha and is made up of 320 Romanian and foreign varieties. The planting distances varied according to the type of rose, as follows: Hybrid tea roses were planted 90 cm apart between rows and 40 cm apart within rows; Floribunda roses at 90 × 70 cm; Shrubs at 1.5 m × 1.5 m; Ground cover at 2.0 m × 2.0 m; and Climbers at 1.5 m × 1.5 m.

From these cultivars, 9 were created as part of the local research of HRS (denoted with *) and they could be classified according to their rose types, as follows: Miniature ('Cri-Cri'), Floribunda ('Foc de tabără'*, 'Jimini Cricket', 'Marie Elisabeth', 'Rosatop'*), Thea Hybrida ('Amalia', 'Baccara', 'Chrysler Imperial', 'First Red', 'Fortuna', 'Fruhrot', 'Galia', 'Orange Ace'*, 'Romana'*, 'Romstar'*, 'Yakimour'), Shrub ('Don Juan', 'Fire Glow', 'Heidekind', 'Pasiune Mov'*, 'Red Candy'*, 'Rosadoll'*, 'Royal Bassino', 'The Fairy Red', and 'Zburlici'*).

Anthocyanin extraction

To extract the anthocyanin and non-anthocyanin fractions from the raw rose plants, a protocol based on recent studies has been adapted (Balík *et al*., 2013; Nile *et al*., 2015). This extraction consisted of the treatment of dried rose petals with acidified methanol (using HCl in a 0.3% concentration, *v/v*) to prevent anthocyanin degradation, followed by mixing using a homogenizer (Miccra D-9 KT Digitronic, Bergheim, Germany). Subsequently, the plant material has been re-extracted until the residue showed no more coloration, during the night, at 4 °C, and in dark.

The primary, colored extract was subjected to filtration through multiple cotton filters, and then concentrated under vacuum at 35 °C using a rotary evaporator, obtaining the anthocyanin and non-anthocyanin fractions. From this, the less polar fractions were eliminated through liquid-liquid extraction, using ethyl acetate. The remaining aqueous fraction was purified using an Amberlite XAD-7 column (1×0.5 cm), pre-conditioned with 6 volumes of 0.3% TFA (trifluoroacetic acid in H_2O, *v/v*). In this step, sugars, pectin, and other impurities have been eliminated. The remaining anthocyanins and procyanidins were eluted with 4 volumes of MeOH (with 0.3% TFA, *v/v*) and purified on a Sephadex LH-20 column (2.5×0.5 cm), preconditioned with 10 volumes of a 8:2 mix of H_2O:MeOH (with 0.3% TFA, *v/v*), obtaining the anthocyanin-rich fraction (ARF). ARF was completed to a final volume of 5 mL with distilled water, filtered through 0.45 μm microfilter, and immediately subjected to the described analyses.

HPLC analysis

The chromatographic separation and identification were accomplished using a simple HPLC (high-performance liquid chromatography) technique, on a HPLC Shimadzu Prominence system equipped with a LC-20 AT binary pump, a DGU-20 A3 degasser, and an SPD-M20 UV-VIS detector. In this reverse-phase HPLC method, the separation was achieved using a Luna Phenomenex C-18 column (5 μm, 25 cm × 4.6 mm). Mobile phase consisted of 4.5% formic acid (FA) in ultrapure water (solvent A) and acetonitrile (solvent B), and the elution was done using a gradient, as follows: 0-9 min (constant 10% of solvent B); 9-17 min (linear growth to 12% of solvent B); 17–30 min (linear growth to 25% of solvent B); 30-50 min (constant 90% of solvent B). Liquid flow was set to 0.8 mL/min and the column temperature was set to 35 °C. Separate identification of anthocyanins was applied with UV detection at a wavelength of 520 nm, according to previous determinations (Bunea *et al*., 2011). For the quantification of individual anthocyanins, an analytical standard of cyanidin-3-*O*-glucoside (product no. 1151935, Sigma-Aldrich) was used (at 520 nm), with linear concentrations of the calibration curve in the range 2.5-500 μg/mL ($R^2 > 0.998$).

Total phenolic content

The total phenolic content (TPC) of rose petals was determined using an adaptation of the Folin-Ciocâlteu (FC) colorimetric method (Saeed *et al*., 2012; Shameh *et a*l., 2018). In this method, 2M of FC reagent was diluted 1:10 with distilled water. The samples were mixed with diluted reagent for a total of 19 minutes. After this, 2 mL of 7.5% sodium carbonate solution was added to neutralize and alkalinize the reaction medium and form the reduced. The absorption of the blue-stained polyphosphomolybdate species that were obtained were read after two hours at 750 nm. Preparation of the calibration curve was made by mixing 2.5 mL of diluted FC reagent with gallic acid solution of different concentrations: 0.2, 0.4, 0.6, 0.8, 1.0, 2.0 µM/mL, and determination in the same conditions. The results were expressed as mg of gallic acid equivalents per 100 g of fresh sample (mg GAE/100 g FW).

Total flavonoid content

The total flavonoid content (TFC) of rose petal petals was determined using a colorimetric method based on aluminum chloride and sodium nitrite (Dewanto *et al*., 2002; Saeed *et al*., 2012). After the samples were diluted with distilled water to a final volume of 5 mL, 0.3 mL of 5% $NaNO_2$ was added, and the mixture was incubated for 5 min. Then, 0.3 mL of $AlCl_3$ 10% solution was added and after 6 minutes another 2 mL of 1N NaOH. Finally, 2.4 mL of water was added, the solution was well homogenized, and the absorbance of the complex was measured at 510 nm. Preparation of the calibration curve was made by applying the same protocol to quercetin as a standard, with different concentrations: 100, 150, 200, and 250 mg/L. TFC was expressed as mg quercetin equivalents per 100 g of fresh sample (mg QE/100 g FW).

In vitro antioxidant activity

A variant of the DPPH method was used for the determination of *in vitro* antioxidant activity (Brandwilliams *et al*., 1995; Sharma *et al*., 2015). A stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of 70% ethanol and the solution was stored at -20 °C until use. The DPPH solution for the reaction was prepared by diluting the stock solution with 70% ethanol to give an absorbance of 1.1 ± 0.02 units at 522 nm. Firstly, 1950 µl of the working DPPH solution was mixed with 50 µl extract, and the mixture was incubated for 20 min. A control solution was prepared in parallel to which the DPPH solution and 50 µl of 70% ethanol were added. The absorbance was measured using an UV-Vis spectrophotometer, and the results were expressed as µmol Trolox equivalents/g fresh substance (µmol TE/g FW).

Statistical analysis

The samples were analyzed in three replicates and the results were expressed as mean ± standard deviation. The results were statistically analyzed using IBM SPSS Statistics 23, and differences were calculated according to Duncan's test ($p < 0.05$).

Results and Discussion

Total anthocyanin content

According to the summed individually quantified anthocyanins, the values ranged from 20.26 to 1206.84 mg/100 g fresh petals, with the varieties 'Jimini Cricket' and 'Amalia' representing these limits, as shown in Figure 1. After 'Amalia', the varieties 'Foc de tabără', 'Fruhrot', and 'First Red' followed as being the richest in anthocyanins, presenting contents of 948.351 mg/100 g FW, 882.92 mg/100 g, and 719.04 mg/100 g, respectively. The lowest values were recorded for the varieties 'Jimini Cricket', 'Cri-Cri', 'Fortuna', and 'Zburlici', with the values of 20.267 mg/100 g FW, 80.106 mg/100 g, 82.697 mg/100 g, and 84.969 mg/100 g.

These results are much higher in comparison to the results of Yang and Shin, who obtained 0.61-502.64 mg/100 g FW (Yang and Shin, 2017).

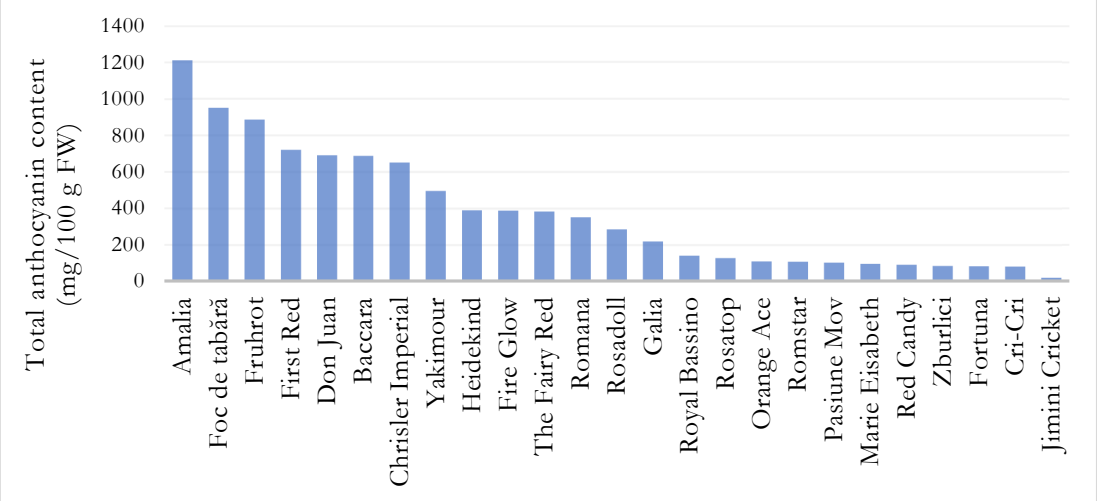

Figure 1. Graphical representation of the total anthocyanin content (as mg/100 g FW) identified for the rose cultivars, shown in decreasing order

One of the most important factors necessary for the generation of the well-known hues of rose petals is related to the concentration of intracellular anthocyanins, which in turn has important commercial implications, given the ornamental value of the plant (Lee *et al*., 2011; Schulz *et al*., 2016). Moreover, the specific composition of the pigments, namely anthocyanins and carotenoids, dictates the final hue of the roses. Specifically, the accumulation of anthocyanins in rose varieties induces petals with red and pink colors (Wang *et al*., 2023).

The qualitative and quantitative profile of anthocyanin and other polyphenolic species in rose petals is in close correlation with the stage of development and the intensity of flower coloration. For example, it was shown that the anthocyanin content is low during the budding phase and much higher when the flower is fully open, yet it starts to decrease during senescence. Schmitzer et al. determined that the anthocyanin content in red rose petals grew from the budding stage to the partially open and fully open stages, and then decreased during flower senescence (Schmitzer *et a*l., 2009, 2010).

Anthocyanin identification and quantification

High-performance liquid chromatography (HPLC) was used for the identification of anthocyanins. On the basis of the retention times, by comparison with standards and literature data, 9 anthocyanins were identified (presented in decreasing order of their amount): cyanidin-caffeoyl-glucoside, cyanidin-coumaroyl-glucoside, cyanidin-glucoside, pelargonidin-glucoside, cyanidin, delphinidin, peonidin-arabinoside, pelargonidin, and peonidin (Table 1).

Table 1. The results of individual anthocyanin content, identified using HPLC with UV-VIS detection (520 nm), expressed as mg anthocyanin/100 g fresh weight

No.	Cultivar	Cya-Caf-G	Cya-Cou-G	Cya-G	Pel-G	Cya	Del	Pel-A	Pel	Peo
1	Amalia	863.9	134.4	60.99	85.43	31.91	30.16	ND*	ND	ND
2	Baccara	338.2	231.5	13.44	39.83	19.66	42.69	ND	ND	ND
3	Chrisler Imperial	548.0	24.61	69.42	4.148	ND	ND	ND	ND	ND
4	Cri-Cri	22.47	48.72	4.803	4.097	ND	ND	ND	ND	ND
5	Don Juan	570.2	33.11	72.48	4.339	ND	ND	ND	ND	ND
6	Fire Glow	114.3	226.8	10.82	3.237	7.948	10.61	24.45	1.810	ND
7	First Red	618.4	8.207	40.49	42.90	4.201	3.737	ND	ND	ND
8	Foc de tabără	545.0	330.8	18.18	15.31	18.18	20.78	ND	ND	ND
9	Fortuna	9.610	65.55	4.716	2.817	ND	ND	ND	ND	ND
10	Fruhrot	757.1	57.10	18.47	31.13	8.916	3.211	6.971	ND	ND
11	Galia	51.29	123.9	4.481	2.402	21.20	10.62	2.108	2.062	ND
12	Heidekind	296.3	40.74	12.06	28.41	4.419	6.578	1.803	ND	ND
13	Jimini Cricket	6.166	5.401	1.865	1.220	2.590	1.388	ND	ND	ND
14	Marie Eisabeth	81.64	4.654	8.797	ND	ND	ND	ND	ND	ND
15	Orange Ace	29.29	68.33	6.001	2.647	ND	ND	ND	ND	ND
16	Pasiune Mov	95.54	1.266	2.048	ND	ND	ND	ND	ND	ND
17	Red Candy	75.38	4.068	8.721	1.391	1.356	ND	ND	ND	ND
18	Romana	232.0	61.62	9.929	9.815	9.742	12.56	4.892	3.175	ND
19	Romstar	85.69	15.07	2.084	2.233	1.500	ND	ND	ND	ND
20	Rosadoll	254.6	16.94	3.796	3.230	4.779	ND	ND	ND	ND
21	Rosatop	113.3	2.942	11.50	ND	ND	ND	ND	ND	ND
22	Royal Bassino	123.8	7.454	2.326	3.976	ND	ND	ND	ND	ND
23	The Fairy Red	269.4	47.31	14.44	14.74	10.31	5.627	3.564	16.30	2.916
24	Yakimour	444.6	7.786	21.32	19.80	ND	ND	ND	ND	ND
25	Zburlici	65.73	3.626	11.67	1.848	2.086	ND	ND	ND	ND

*Notes: ND – not detected. Anthocyanins: Cya: cyanidin; Cya-G: cyanidin-glucoside; Cya-Caf-G: cyanidin-caffeoyl-glucoside; Cya-Cou-G: cyanidin-coumaroyl-glucoside; Del: delphinidin; Pel: pelargonidin; Pel-A: pelargonidin-arabinoside; Pel-G: pelargonidin-glucoside; Peo: peonidin.

As expected, there was a clear variation in the anthocyanin profile of the rose varieties, both qualitatively and quantitatively. The only sort in which all 9 anthocyanins were identified was 'The Fairy Red', and the other 8 compounds (excepting peonidin) were identified successfully in the varieties 'Fire Glow', 'Galia', and 'Romana'.

Cyanidin-caffeoyl-glucoside was identified and quantified as being the main anthocyanin in the petals, presenting a wide range, from 6.166 mg/100 g FW (in 'Jimini Cricket' variety) to 863.9 mg/100 g FW (in 'Amalia' variety). This metabolite showed maximum values in all the varieties excepting 'Fire Glow', 'Galia', 'Fortuna', and 'Orange Ace', in which cyanidin-coumaroyl glucoside is more abundant. Cyanidin-glucoside and pelargonidin-glucoside were also abundant, with the maximum values of 72.48 mg/100 g and 85.43 mg/100 g, respectively. Peonidin was present only in 'The Fairy Red' variety, however, in a low concentration (2.916 mg/100 g), while cyanidin, delphinidin, pelargonidin, and pelargonidin-arabinoside were less representative in the selected varieties. The presence of cyanidin and pelargonidin mono- and di-glucosides, as well as the simple aglycones, has been previously determined for rose petals (Schulz *et al*., 2016). Several authors have observed that pelargonidin-3,5-di-*O*-glucoside and cyanidin-3,5-di-*O*-glucoside represent the major anthocyanins in rose petals (Wang *et al*., 2023), along with cyanidin- and pelargonidin-3-*O*-glucosides (Wan *et al*., 2019).

Total phenolic content

The total phenolic content (TPC) of the studied rose petals varied from 208 ± 23 to 2600 ± 452 mg GAE/100 g FW, as it can be observed in Table 2. From these, the highest value was identified for 'Amalia', followed by 'Bonfire' (2502 ± 397 mg GAE/100 g FW), *Fruhrot* (2464 ± 671 mg GAE/100 g), 'First Red' (2384 ± 453 mg GAE/100 g), and 'Don Juan' (2006 ± 37 mg GAE/100 g). On the other hand, the varieties with the lowest values were 'Jimini Cricket', followed by 'Red Candy' (237 ± 34 mg GAE/100 g FW), 'Cri-Cri' (244 ± 36 mg GAE/100 g), and 'Zburlici' (287 ± 21 mg GAE/100 g), showing close values. The maximal values are similar to previous determinations. For example, Ge and Ma determined values of 2087.43 ± 17.37 mg GAE/100 g FW for the methanolic extracts obtained from rose petals (Ge and Ma, 2013). Similarly, Selvi et al. have determined the TPC value of 1290 mg GAE/100 g for dried rose petals, which were extracted with methanol/water beforehand (Selvi *et al*., 2020). Other studies established smaller maximal values for the methanolic extracts of rose petals, such as 296.60 µg GAE/mg of extract (Pal et al., 2018); however, these values are still within the range of our determinations.

Total flavonoid content

The values for the total flavonoid content (TFC), presented in Table 2 as well, have ranged between 160 ± 23 mg QE/100 g FW (for 'Zburlici') and 705 ± 49 mg QE/100 g (for 'Amalia'). In correlation with the values of total anthocyanin content, the richest cultivars in flavonoids were 'Amalia', 'Foc de tabără', 'Fruhrot', and 'First Red'. The results of our determinations were higher compared to a recent literature study that obtained maximum values of 531.54 and 392.69 mg/100 g FW for ethanolic extracts of the petals of some edible roses (*Rosa hybrida* spp.) (Yang and Shin, 2017). Similarly, Shameh *et al*. (2018) obtained values of 0.61–0.82 mg QE/g DW for the extracts of the petals from six *Rosa* species (Shameh *et al.*, 2018).

Table 2. The results of total phenolic content (TPC, as mg GAE/100 g FW), total flavonoid content (TFC, as mg QE/100 g FW), and antioxidant activity through the DPPH method (as µmol TE/g FW)

No.	Cultivar	TPC	TFC	DPPH
1	Amalia	2600±45^{a}	705±49^{a}	1403±301^{a}
2	Baccara	1765±176^{c}	541±78^{cd}	1274±201^{ab}
3	Chrisler Imperial	1975±245^{bc}	543±67^{cd}	1297±201^{a}
4	Cri-Cri	244±36^{i}	221±13^{hi}	402±69^{fg}
5	Don Juan	2006±378^{bc}	598±75^{bc}	1305±231^{a}
6	Fire Glow	1117±234^{e}	387±56^{fg}	1198±104^{ab}
7	First Red	2384±453^{ab}	602±56^{bc}	1302±205^{a}
8	Foc de tabără	2502±397^{a}	700±76^{a}	1400±234^{a}
9	Fortuna	221±67^{i}	207±43^{hi}	307±77^{g}
10	Fruhrot	2464±671^{a}	657±57^{ab}	1375±167^{a}
11	Galia	782±79^{efgh}	231±73^{hi}	675±91^{def}
12	Heidekind	1231±201de	412±54^{ef}	1241±121^{ab}
13	Jimini Cricket	208±23^{i}	213±26^{hi}	304±37^{g}
14	Marie Eisabeth	302±43^{i}	162±34^{i}	407±38^{fg}
15	Orange Ace	551±65^{fghi}	231±28^{hi}	602±43^{ef}
16	Pasiune Mov	487±43^{ghi}	208±29^{hi}	542±56^{efg}
17	Red Candy	237±34^{i}	236±53^{hi}	398±56^{fg}
18	Romana	1002±167^{ef}	345±78^{fg}	1005±227^{bc}
19	Romstar	401±24^{hi}	176±41^{i}	501±91^{fg}
20	Rosadoll	954±78^{ef}	332±66^{fg}	905±79^{cd}
21	Rosatop	553±78^{fghi}	228±61^{hi}	653±78^{def}

22	Royal Bassino	885±68^{cfg}	305±37^{gh}	804±81^{cdc}
23	The Fairy Red	1589±123^{cd}	491±48de	1263±227^{ab}
24	Yakimour	1702±346^{c}	505±28^{cd}	1286±131^{a}
25	Zburlici	287±21^{i}	160±23^{i}	406±47^{fg}

Notes: Results were expressed as average ± standard deviation (n = 3). Different superscript letters on the same column indicate significant statistical difference between total anthocyanin content, based on Duncan's test ($p < 0.05$).

In vitro antioxidant activity

Finally, the *in vitro* antioxidant activity for each petal extract was determined by the DPPH method, and the results are presented in Table 2. The 'Amalia' variety showed the highest amount of total anthocyanins, polyphenols, and flavonoids, yet it also showed the highest antioxidant activity of 1403 ± 301 μmol TE/g FW, followed by 'Foc de tabără' with a very close value (1400 ± 234), 'Fruhrot' (1375 ± 167), 'Don Juan' (1375 ± 167), and 'First Red' (1302 ± 205). The lowest antioxidant activity was recorded in 'Jimini Cricket' (304 ± 37) and 'Fortuna' (307 ± 77) varieties. The results are similar to those presented in some recent studies. As an example, Trinh et al. obtained higher values of 873.4–1635.6 μmol TE/g for rose extracts (Trinh et al., 2018). Moreover, it was observed that the water and methanolic fractions of red rose petals extracts showed the lowest IC_{50} values (representing the highest antioxidant activity) using the DPPH assay, specifically 25.66 μg and 33.73 μg (Pal *et al*., 2018).

Conclusions

In this study, 25 different rose sorts were collected and analyzed through different methods. Firstly, the anthocyanin compounds were extracted, purified, separated, and identified using an adapted HPLC method with UV-VIS detection. The anthocyanin-rich extracts were also assessed for the total content in phenolic, flavonoid content, as well as for their *in vitro* antioxidant potential through the DPPH method. A total of nine anthocyanins were identified in the samples: cyanidin-caffeoyl-glucoside (the most abundant), cyanidin-coumaroyl-glucoside, cyanidin-glucoside, pelargonidin-glucoside, cyanidin, delphinidin, peonidin-arabinoside, pelargonidin, and peonidin. The 'Amalia' cultivar showed the highest anthocyanin content (1206.841 ± 103 mg/100 g FW), while 'Jimini Cricket' showed the lowest (20.267 ± 8.7 mg/100 g FW). The same trend was observed for the other determinations.

Authors' Contributions

Conceptualization A.B., I.C.P., G.R., R.S. and A.P.; Sampling G.R., I.C.P. and A.B.; methodology A.B., C.I.B. and A.P.; investigation A.B., I.C.P., A.P. and A.N.; data curation A.B., A.P., C.I.B., R.S. and A.N.; writing—original draft preparation A.N., writing—review and editing A.B., I.C.P., G.R., A.P., C.I.B, R.S. and A.P.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

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

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