STUDIES ON THE PHENOLIC CONTENT OF RED SENESCENT GRAPEVINE LEAVES - A SUSTAINABLE SOURCE OF BIOACTIVE COMPOUNDS

STUDII PRIVIND CONŢINUTUL FENOLIC AL FRUNZELOR ROŞII SENESCENTE DE VIŢĂ DE VIE – SURSĂ SUSTENABILĂ DE COMPUŞI CU ROL BIOLOGIC ACTIV

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Abstract. *In order to identify new sustainable sources of bioactive compounds for food, pharmaceutical and cosmetic industries, anthocyanin, proanthocyanidin and total phenolic contents of red senescent leaves of 47 grapevine cultivars and chromatic parameters of their alcoholic extracts were evaluated. Leaves were picked between two to eight weeks after grape harvest, the remaining chlorophyll and carotenoid amounts being also quantified. Red grapevine leaves showed high antioxidant activity (% scavenged DPPH), proportionally correlated with the concentration of anthocyanins and total phenolic compounds. Chlorophyll and carotenoid content of red leaves was low, without exceeding 1.56 mg/g and 0.92 mg/g dry weight, respectively. High amounts of antioxidant anthocyanins, proanthocyanidins and total phenolic compounds, as well as the chromatic diversity of the obtained extracts, justify the use of senescent red grapevine leaves as raw material in the production of valuable dietary supplements.*
Key words: antioxidant activity, anthocyanins, chromatic parameters, grapevine leaves, proanthocyanidins

Rezumat. *În vederea identificării unor surse noi şi sustenabile de compuşi cu rol biologic activ pentru industriile alimentară, farmaceutică şi cosmetică, a fost evaluat conţinutul de antociani, proantocianidine şi compuşi fenolici totali din frunzele roşii senescente provenind de la 47 de soiuri de viţă de vie, precum şi caracteristicile cromatice ale extractelor alcoolice obţinute. Frunzele au fost colectate după două până la opt săptămâni de la recoltarea strugurilor, cantităţile remanente de clorofilă şi carotenoizi fiind de asemenea cuantificate. Extractele de frunze au prezentat o activitate antioxidantă ridicată (% DPPH), direct corelată cu prezenţa antocianilor şi compuşilor fenolici totali. Conţinutul de clorofile şi carotenoizi a fost redus, ajungând până la 1,56 mg/g şi respectiv, 0,92 mg/g masă uscată. Cantităţile mari de compuşi fenolici cu activitate antioxidantă ridicată, precum şi diversitatea cromatică a extractelor obţinute, justifică utilizarea frunzelor roşii senescente de viţă de vie ca materie primă în obţinerea alimentelor funcţionale.*
Cuvinte cheie: activitate antioxidantă, antociani, frunze roşii, parametri cromatici, proantocianidine

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INTRODUCTION

Red colouring of autumn leaves is a phenomenon that affects the normally green leaves, occurring mostly at grapevine cultivars with dark coloured grapes, varying depending on several factors such as: chlorophyll concentration (higher chlorophyll concentrations give leaves a purple to brown appearance), pH (basic pH in vacuoles results in blue coloration, whereas more acidic pH results in red), type of anthocyanins, co-pigmentation, glycosylation or the presence of metal ions. Anthocyanin synthesis in senescent leaves is thought to reduce light stress and provide protection against oxidation (Steyn *et al*., 2002). Also, red leaves may appear as a result of nutrient deficiency, viral or phytoplasma infection during the growing season, the symptoms being easily recognised.

Research conducted in recent years reported a high number of therapeutic properties of polyphenol rich extracts of red vine leaves. *In vitro* studies have shown vasorelaxing, antibacterial and anti-inflammatory activity, and particular antioxidant properties of grapevine foliar polyphenols (Katalinić *et al*., 2009).

The objective of this research was to assess new and sustainable sources of compounds with sanogenous potential, for use in food, pharmaceutical and cosmetic industries. Since phenolic compounds are considered responsible for various health benefits of red vine leaves, their content needs to be evaluated.

MATERIAL AND METHOD

The research has been carried out on red leaves of 47 grapevine cultivars for wine (31) and table grapes (16), growing in the Ampelographic Collection of the University of Agricultural Sciences and Veterinary Medicine Iaşi, N-E of Romania (27°53' E and 47°09' N). Red vine leaves were picked by hand at they phenolic maturity, between two to eight weeks after the grape harvest (depending on cultivar) as previously reported by Schneider *et al*. (2008). Phenolic compounds were extracted from grinded air-dried leaves (10 - 12 % moisture), with 0.1% HCl (v/v) in 96% ethanol (1:30, w/v), 20 h at 20 °C, in darkness. Chlorophylls and carotenoids were extracted with pure acetone (1:20, w/v), according to CIS (2011).

Total phenolic compounds (OIV, 2012), monomeric anthocyanins (Lee *et al*. 2005), total proanthocyanidins (Caceres-Mella *et al*., 2013), photosynthetic pigments (CIS, 2011) and the colour components (Glories, 1984), were determined by an UV-vis Shimadzu 1700 Pharmaspec spectrophotometer. Antioxidant activity of leaf extracts was assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, at λ = 517 nm. Stock solution: 2.36 mg DPPH in 100 mL ethanol. The used formula was: Inhibition (%) = [(Absorbance T_0 − Absorbance T_{30})/Absorbance T_0] × 100.

RESULTS AND DISCUSSIONS

Analysis of the chromatic parameters can give information about the quality characteristics of a product and may be associated later with consumer acceptance. Chlorophyll (Chl) and carotenoid content of red leaves was low, specific for the final phenophase of annual cycle of grapevine vegetation. The percentage of red in the formation of extract colour was high (due to anthocyanins), along with yellow nuances (due to carotenoids). Red leaves of Fetească neagră cv. showed the highest colour intensity (62.08) (tab. 1).

Table 1

Photosynthetic pigments and phenolic compounds content of red leaf extracts and their colour components

No.	Cultivar	Photosynthetic pigments (mg/g)			TPC (g GAE/ 100 g)	A_{280}	PA (mg Cat / 100 g)	ANT (mg CE/ 100 g)	Colour components				
		Chl a	Chl b	Car					%Y	%R	%B	CI	H
0	1	2	3	4	5	6	7	8	9	10	11	12	13
1	Alicante bouschet	0.28	0.19	0.28	4.27	75.95	70.91	355.31	60.31	35.26	4.43	11.74	1.71
2	Alidor	0.35	0.15	0.05	1.52	31.75	50.79	82.92	67.00	27.64	5.36	7.09	2.42
3	Alvarna	0.11	0.10	0.15	3.22	73.85	62.28	59.25	78.54	17.14	4.32	6.71	4.58
4	Andre	0.66	0.43	0.92	5.34	96.10	80.91	279.29	33.24	63.97	2.79	40.88	0.52
5	Aramon	0.26	0.20	0.05	2.78	64.15	57.69	68.97	79.92	15.19	4.89	7.57	5.26
6	Babească neagră	0.29	0.17	0.25	3.77	44.75	62.62	122.74	50.38	45.58	4.04	10.64	1.11
7	Balada	0.91	0.45	0.22	4.63	97.95	84.71	110.63	47.46	48.50	4.05	22.99	0.98
8	Bastard de Magaraci	0.57	0.31	0.73	5.60	106.05	76.18	354.72	32.53	65.00	2.47	39.26	0.50
9	Batută neagră	0.38	0.28	0.45	6.89	103.70	88.37	290.54	41.79	55.82	2.39	25.10	0.75
10	Black rose	0.34	0.21	0.28	5.51	63.40	77.11	298.06	51.66	44.37	3.97	13.86	1.16
11	Blauerzweigelt	0.44	0.02	0.10	5.41	74.25	72.28	213.33	39.97	56.74	3.30	23.67	0.70
12	Burgund mare	0.75	0.32	0.57	4.14	66.35	69.18	205.40	41.43	55.48	3.09	28.46	0.75
13	Cabernet Sauvignon	0.86	0.39	0.39	4.30	48.65	65.72	184.52	47.74	48.29	3.97	23.67	0.99
14	Cardinal	0.29	0.17	0.04	2.47	31.35	35.04	50.51	77.46	17.55	4.99	6.21	4.41
15	Cinsaut	0.95	0.45	0.45	5.29	76.95	75.38	203.31	48.55	47.51	3.93	26.94	1.02
16	Coarnă neagră	0.97	0.42	0.07	2.45	33.20	49.43	93.10	85.53	8.76	5.72	13.82	9.77
17	Codană	0.44	0.19	0.10	3.60	50.85	58.25	91.84	66.11	29.47	4.42	9.50	2.24
18	Dimiat	0.44	0.23	0.04	1.57	30.75	33.89	55.52	72.38	21.95	5.67	8.29	3.30
19	Dodrelabi	0.94	0.49	0.16	3.31	58.65	57.91	225.02	64.59	29.53	5.87	17.37	2.19
20	Durif	0.64	0.30	0.14	2.73	44.65	44.57	89.34	63.74	30.59	5.67	12.52	2.08
21	Fetească neagră	0.77	0.48	0.42	7.21	131.85	88.45	408.75	34.28	63.18	2.55	62.08	0.54
22	Gamay beaujolais	0.13	0.14	0.54	5.99	77.65	74.37	415.21	63.89	32.50	3.60	11.66	1.97
23	Gelu	0.25	0.12	0.11	1.61	25.00	7.09	120.65	69.05	26.39	4.56	5.04	2.62
24	Kismis negru	1.07	0.42	0.42	6.33	130.60	82.16	198.99	48.91	18.73	32.36	35.88	2.61

Table 1 – continuation

0	1	2	3	4	5	6	7	8	9	10	11	12	13
25	Merlot	0.90	0.44	0.23	4.89	90.15	56.94	163.23	74.03	19.35	6.62	16.02	3.83
26	Michele Palieri	0.41	0.29	0.51	6.09	129.55	80.33	189.41	57.34	37.79	4.87	16.62	1.52
27	Milcov	1.04	0.45	0.49	5.78	101.05	70.09	172.83	50.22	45.69	4.10	27.82	1.10
28	Mioriţa	0.44	0.25	0.25	2.75	55.45	41.37	42.16	69.31	23.58	7.11	8.44	2.94
29	Moldova	0.19	0.51	0.81	7.23	127.60	83.43	298.85	40.37	56.22	3.41	37.28	0.72
30	Muscat de Hamburg	0.69	0.34	0.58	2.06	26.00	38.25	149.46	76.87	16.61	6.51	9.21	4.63
31	Muscat timpuriu de Bucureşti	1.06	0.50	0.45	4.77	70.75	63.10	163.23	48.01	48.21	3.78	34.39	1.00
32	Napoca	0.76	0.43	0.29	2.49	49.75	29.21	129.83	51.57	43.64	4.79	18.79	1.18
33	Negru de Căuşani	0.36	0.34	0.66	6.59	118.45	74.81	233.73	51.29	44.51	4.21	21.39	1.15
34	Negru de Dragăşani	0.99	0.43	0.29	2.10	51.05	39.52	153.21	55.23	40.24	4.53	21.62	1.37
35	Negru vârtos	0.74	0.33	0.23	4.08	48.65	66.18	125.66	54.97	40.44	4.60	17.41	1.36
36	Novac	0.77	0.39	0.52	6.54	98.15	84.59	282.63	48.34	47.38	4.28	23.81	1.02
37	Oporto	1.03	0.46	0.27	3.48	67.45	49.06	95.18	61.74	33.08	5.18	23.55	1.87
38	Pinot noir	0.09	0.09	0.29	1.87	35.95	33.90	162.81	31.24	67.05	1.71	12.29	0.47
39	Princess	1.10	0.28	0.68	4.70	96.10	66.32	198.30	50.97	44.78	4.25	35.04	1.14
40	Purpuriu	0.35	0.11	0.22	3.07	71.80	57.79	172.00	75.58	18.58	5.84	5.65	4.07
41	Roz de Miniş	0.65	0.31	0.15	5.08	90.80	60.55	133.49	75.02	18.91	6.07	11.21	3.97
42	Someşan	0.20	0.08	0.03	2.37	36.25	50.10	200.40	68.78	26.59	4.63	4.10	2.59
43	Splendid	0.44	0.41	0.92	6.92	126.60	74.23	301.57	20.86	76.55	2.59	43.25	0.27
44	Sultanina	0.64	0.28	0.15	3.12	34.40	55.38	59.70	59.43	35.82	4.75	13.26	1.66
45	Transilvania	0.36	0.14	0.04	1.97	25.10	9.74	129.83	69.78	24.70	5.53	5.79	2.83
46	Trollinger	0.31	0.14	0.12	1.91	21.95	15.38	101.11	74.07	19.06	6.88	5.09	3.89
47	Vulpea	0.31	0.25	0.75	4.15	56.70	59.40	327.73	29.00	68.89	2.12	32.14	0.42

Note: Chl a - chlorophyll a; Chl b - chlorophyll b; Car - carotenoids (xanthophylls and carotenoids); TPC (g GAE/ 100 g) - total phenolic content (g Gallic acid equivalent/ 100 g dry weight); A_{280} - absorbance at 280 nm (estimation of total phenols); PA (mg Cat / 100 g) - proanthocyanidins (g catechin equivalent / 100 g); ANT (mg CE / 100 g) - monomeric anthocyanins (mg cyanidin-3-glucoside equivalent/100 g); %Y, %R, %B - percentages of yellow, red and blue in extract colour; CI - colour intensity (sum of absorbance at 620 nm, 520 nm and 420 nm); H - hue (ratio of optical densities at 420 nm and 520 nm).

A high concentration of anthocyanins was found in leaves of "teinturier" cultivars Gamay beaujolais and Alicante bouschet, and of Romanian cultivars Fetească neagră and Vulpea (327 - 415 mg CE/100 g), while total phenolic content of red leaves varied from 1.52 to 7.23 g GAE/100 g. Data are consistent with those reported by Schneider *et al*. (2008) and Ignat *et al.* (2016).

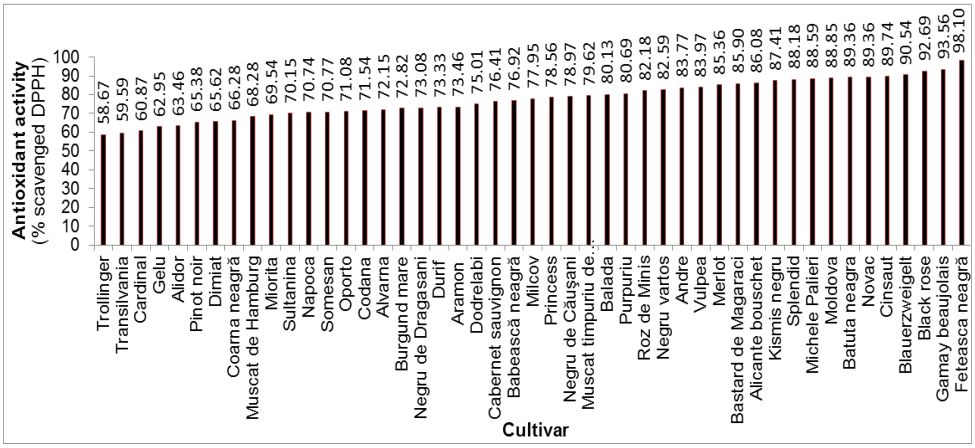

Fig. 1 Antioxidant activity of red senescent grapevine leaves

Antioxidant activity of leaf extracts was high, between 58.67 and 98.10 % scavenged DPPH (fig. 1), correlated with the abundance of anthocyanins (r = 0.7709), proanthocyanidins (r = 0.8621) and total phenolic content (r = 0.8915).

Table 2

The correlation of experimental data

Parameters	**Chl a**	**Chl b**	**Car**	**AA**	**ANT**	**PA**	**TPC**
Chl b	0.7607	-					
Car	0.1436	0.4574	-				
AA	0.1281	0.3245	0.5529	-			
ANT	-0.0724	0.2002	0.6756	**0.7709**	-		
PA	0.1929	0.3808	0.5510	**0.8621**	0.6038	-	
TPC	0.1682	0.4324	0.6606	**0.8915**	**0.7253**	**0.8703**	-
A_{280}	0.2002	0.4697	0.6438	**0.7916**	0.6083	**0.8062**	**0.9069**

Note: Chl a - chlorophyll a; Chl b - chlorophyll b; Car - carotenoids; AA - antioxidant activity; ANT - monomeric anthocyanins; PA - proanthocyanidins; TPC - total phenolic content; A_{280} - absorbance at 280 nm.

A strong correlation was found between anthocyanin and total phenolic content (r = 0.7253) and between proanthocyanidins and total phenolic content (r = 0.8703) of senescent leaves (tab. 2). As shown in our previous studies (Filimon *et al.*, 2015), neither in this cases, no relationship was found between anthocyanin and photosynthetic pigment content of red leaves.

CONCLUSIONS

1. Red senescent grapevine leaves contained high amounts of phenolic compounds, including anthocyanins and proanthocyanidins, which greatly contributed to the strong antioxidant activity of the leaf extracts.

2. Chlorophyll and carotenoid content of red leaves was low, but exerting a distinct influence on the composition of extracts colour.

3. Red senescent grapevine leaves represent a sustainable source of phenolic compounds for food, pharmaceutical and cosmetic industries, further research being necessary in order to identify the main compounds responsible for the related health benefits and to evaluate their real economic potential.

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