

# Carotenoids, Flavonoids, Total Phenolic Compounds and Antioxidant Activity of Two Creeping *Cotoneaster* Species Fruits Extracts

DAN RAZVAN POPOVICIU<sup>1</sup>, TICUTA NEGREANU-PIRJOL<sup>2,1\*</sup>, LUDMILA MOTELICA<sup>3</sup>,  
BOGDAN STEFAN NEGREANU PIRJOL<sup>2</sup>

<sup>1</sup>Ovidius University of Constanta, Faculty of Natural Sciences and Agricultural Sciences, 1 University Alley, Campus, Corp B, 900470, Constanța, Romania

<sup>2</sup>Ovidius University of Constanta, Faculty of Pharmacy, 1 University Alley, Campus, Corp B, 900470, Constanta, Romania

<sup>3</sup>University Politehnica of Bucharest, Faculty of Applied Chemistry and Materials Science, 1-7 Gh. Polizu, 011061, Bucharest, Romania

**Abstract.** *Cotoneaster horizontalis* Decne. and *Cotoneaster microphyllus* Wall. ex Lindl. species are two creeping bushes, commonly used as ornamental plants in gardens and parks. The aim of this paper was to assess the concentrations of some classes of bioactive compounds classes, carotenoids, flavonoids and total phenolic compounds, in fresh fruits of these species. Carotenoids and flavonoids were determined through acetone and methanol extraction followed by spectrophotometry. For total phenolics, methanol extraction and a spectrophotometric Folin-Ciocalteu method was used. The total antioxidant capacity was quantified through photochemiluminescence method by comparison with the standard substance used for calibration, Trolox® as tocopherol analogue (ACL procedure) using Photochem apparatus, Analytik Jena AG, Germany. Average values found in *Cotoneaster horizontalis* and *Cotoneaster microphyllus* fruit tissue were 380.63 mg/kg, respectively 179.63 mg/kg, carotenoids; 8036.07 mg/kg, respectively 6888.06 mg/kg flavonoids; and 16342.06 mg/kg GAE, respectively 18631.35 mg/kg GAE total phenolic compounds. These values are comparable to those found in other wild and cultivated related Rosaceae, including domestic rowans. *Cotoneaster microphyllus* fruits emphasized an increased antioxidant activity (up to 39.69  $\mu\text{mol}$  Trolox equivalent/g dry weight).

**Keywords:** *Cotoneaster horizontalis*, *Cotoneaster microphyllus*, carotenoids, flavonoids, phenolic compounds, antioxidant activity

## 1. Introduction

*Cotoneaster* is a genus of Rosaceae (Amygdaloideae subfamily, Maleae tribe), comprising 70-300 species worldwide. All species are woody (shrubs and small trees), most being native to West China and the Himalayan region. Cotoneasters are now widely grown as ornamental plants throughout the world, due to their attractive evergreen or semi-evergreen foliage and their dense and bright-colour fruits. Some of them are also increasingly common in Romania, including prostrate species, commonly known as “rocksprays”

*Cotoneaster horizontalis* Decne. (wall/rock cotoneaster, rockspray, wall-spray), is a dwarf shrub, less than 1 m tall, with long prostrate branches, numerous lateral twigs (Figure 1). Leaves are small, elliptic, semi-deciduous and glossy with a rounded apex. White-pink flowers are grouped in compact inflorescences and fruits are small, globosely, bright-red pomes. It is native to Western of China, and grown in parks and gardens throughout temperate areas, including Romania. In some areas of the world, it is considered invasive [1].

*Cotoneaster microphyllus* Wall. ex Lindl. (small-leaf/little leaf cotoneaster, rockspray) is a dwarf shrub, less than 1 m tall, with extensive horizontal branches and twigs (Figure 1). It is a native of Tibet,

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\*email: ticuta\_np@yahoo.com

Yunnan, Sichuan and Nepal. There are four varieties: *cochleatus*, *conspicuus*, *glacialis* and *thymifolius*. Ornamental cultivars grown in Romania belong to *Cotoneaster microphyllus* var. *cochleatus*. Leaves are small, elliptic and evergreen, with an obtuse apex. Flowers are white and solitary, or grouped in small clusters. Fruits are small, globosely, bright-red pomes [2].

Cotoneaster fruits are known as mildly toxic, due to their cyanogenic glycoside content. Through digestion, these glycosides are converted to toxic cyanide. However, the concentration is too low to produce effects in adults. A large raw consumption would lead to brain, heart, kidney or liver illness. In some regions, cotoneaster berries are used for jellies or as a colorant. Known folk medicinal uses of *Cotoneaster microphyllus* fruits include, anti-irritant pastes for skin care and oral treatments for biliary dysfunction and irregular menstruation [2]. Also, the fruits of other *Cotoneaster* species have various applications in folk medicine, against infections, vascular diseases, bronchitis or gastritis [3].

The aim of this paper is to determine the concentrations of some key classes of phytochemicals in fresh fruit tissue of *Cotoneaster horizontalis* Decne. and *Cotoneaster microphyllus* Wall. ex Lindl. species.



**Figure 1.** *Cotoneaster horizontalis* Decne. and *Cotoneaster microphyllus* Wall. ex Lindl.

Carotenoids (carotenes, lutein, lycopene, zeaxanthin etc.) are common pigments in plant leaves, flowers and fruits. They are essential for melanin and retinol production, thus, for skin and eye health, while they also possess antitumor properties [4].

There are various subclasses of phenolic and polyphenolic compounds, with protective functions against microbial and fungal pathogens and insects [5]. Among them, flavonoids, phenolic acids, tannins. These compounds are antioxidants, acting as radical scavengers, reducing agents and lipid oxidation inhibitors [6]. Flavonoids (low-mass polyphenolic compounds) are antiviral, antibacterial, antifungal, anti-inflammatory, antiproliferative and antitumor compounds [5].

## 2. Materials and methods

Mature pomes from different individuals from both species were collected in Constanța city, Romania, in October - November months, 2018. Pyrenes (kernels) were manually eliminated and the fruit pulp was ground, using an electrical grinder. Fruit tissue was kept frozen (-20°C), prior to examination. Part of the material was oven-dried for 72 h at 80°C, for determining dry biomass percentage.

For determining total carotenoids content, 0.1 g ground fruit tissue was extracted in 10 mL 80% acetone (triplicate samples for each species). The extract was filtered at normal pressure through Whatman blue band filter paper and the spectrophotometric absorbance was read (using a S106 WPA UV-Vis spectrophotometer) against an 80% acetone blank, at 470 nm, 647 nm and 663 nm of wavelengths [7]. Absorbance values were used to calculate carotenoids concentration, according to the specific trichromatic equations [8].

For flavonoid determinations, a quantity of 1 g ground fruit tissue was extracted in 5 mL methanol and filtered (triplicate samples). 0.5 mL of extract was diluted in 4 mL water and 8 mL methanol mixture, and the spectrophotometric absorbance was read against a methanol blank at 340 nm wavelength [9].

For determining the concentration of total phenolic compounds, a UV-Vis spectrophotometric version of the Folin-Ciocalteu method was used. 0.1 g fruit tissue was extracted in 10 mL methanol and filtered. 1 mL of extract was reacted with 5 mL Folin-Ciocalteu reagent (10%) and 4 mL sodium bicarbonate solution (7.5%) for 30 min. Spectrophotometric absorbance was read against a blank at 765 nm. A calibration curve was prepared, by using different gallic acid concentrations [10 - 12]. Concentrations were expressed as mg/kg dry weight and mg/kg DW gallic acid equivalent (GAE) for total phenolic compounds.

For determining total antioxidant capacity, a quantity of 1 g, respectively 10 g of fine dried fruit powder, was cold-extracted in 40% ethanol (100 mL total volume), at room temperature and darkness, for 12 days, with regular shaking. After decantation, normal pressure filtration and homogenization (Vortex Velp Scientifica, Italy agitator), 10 µL of supernatant were taken for analysis. Each determination lasted 120 s.

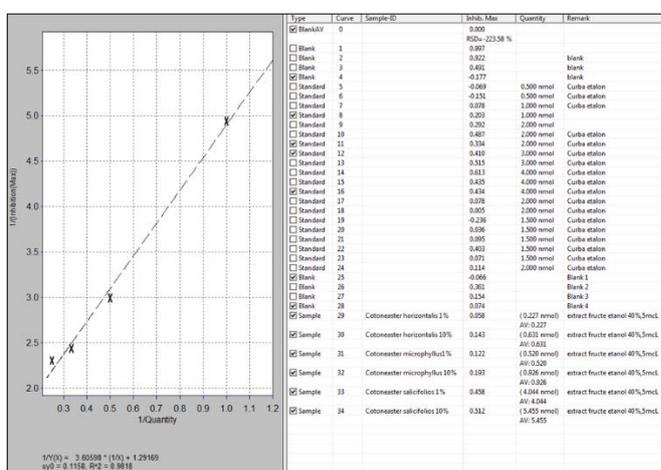
Analyses employed the photochemiluminescence method by ACL (Antioxidative Capacity in Lipid Soluble Substances) procedure Analytik Jena and Photochem apparatus Analytik Jena AG, Germany. Triplicate samples of hydroalcoholic extract were quantified by comparison with the standard substance Trolox®, Hoffman-LaRoche's trade name (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) vitamin E derivative.

For calibration, the standard kit of reagents, Analytik Jena Germany was used: R<sub>1</sub> (dilution solvent), R<sub>2</sub> (buffer reagent), R<sub>3</sub> (photosensitive reagent), R<sub>4</sub> (reagent sized). For the calibration curve (Figure 2) standard solutions containing 0.5, 1.0, 2.0, 3.0 nmol Trolox were measured (suitable for 5 - 30 µL R<sub>4</sub>). By exposure to external radiation from a Hg lamp lined with phosphorus, maximum energy at 351 nm wavelength, the photosensitive reagent produces free radicals in the sample, resulting a photochemical reaction. The superoxide anion radicals produced are partially neutralized by reaction with the antioxidants in the sample. Residual radicals cause luminescence in the detector substance, which can be determined using a photomultiplier tube. The signal produced by the luminescence is traced over 3 min.

The total antioxidant capacity of the selected sample is measured by converting the electrical signal to concentration values and by comparison with the Trolox® standard substance, expressed as nmol Trolox equivalents/sample volume [13, 21, 22]. Samples were prepared, according to Table 1.

**Table 1**  
 Working scheme volumes, µL, [21, 22]

Kit reagent	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	Sample
Blank	2300	200	25	0	0
Calibration curve	2300 – vol.	200	25	vol.	0
Measurement sample	2300 – vol.	200	25	0	ol.



**Figure 2.** Calibration curve for standard substance Trolox® (photochemiluminescence method, ACL procedure, Analytik Jena AG)

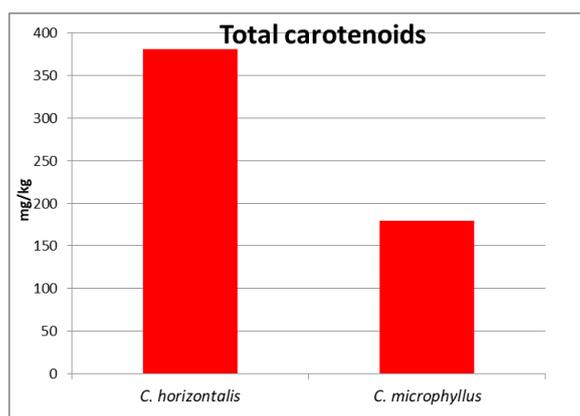
### 3. Results and discussions

Carotenoids concentrations in the selected fruits are shown in Figure 3. Flavonoids and total phenolic compounds level are shown in Figure 4 and Figure 5, respectively.

Considering a 13.1% dry weight percentage, the average amount of total carotenoids was 380.63 mg/kg DW (315.47 - 433.62 mg/kg in individual samples), in *Cotoneaster horizontalis* and 179.63 mg/kg DW (165.57 - 189.83 mg/kg) in *Cotoneaster microphyllus* (Figure 3). Few data concerning the carotenoids content of *Cotoneaster* species are found in literature. An useful comparison standard would be the related genus *Pyracantha* containing over 65 mg/kg carotenoids in *Pyracantha angustifolia* [14], and more than 22 mg/kg in *Pyracantha crenulata* species (known as a valuable medicinal plant, powdered dried fruits used in the treatment of bloody dysentery) [15, 19].

*Sorbus* (the genus comprising rowanberries and service-trees), is also a related genus, in the Maleae tribe, with similar fruits. A comprehensive research on cultivated rowan varieties found a total carotenoid content of 39 - 2659 mg/kg [6].

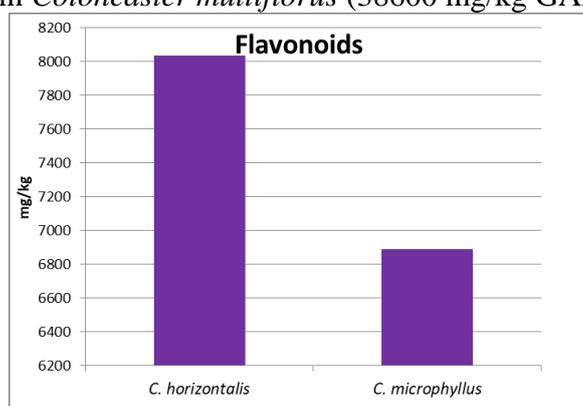
An average of 8036.07 mg/kg DW of flavonoids (4,457 - 10,940 mg/kg) was found in *Cotoneaster horizontalis* fruit tissue. *Cotoneaster microphyllus* fruit tissue contained 6888.06 mg/kg DW flavonoids (3444 - 10332 mg/kg), Figure 4. These values are similar to those found by Mohamed et al. in *Cotoneaster horizontalis* fruits (6800 mg/kg) [1], lower than in *Cotoneaster multiflorus* fruits (53700 mg/kg) [3].



**Figure 3.** Total carotenoids concentration in selected *Cotoneaster* species fruits (average values; mg/kg)

Fruits of *Sorbus* species contain 435 - 37000 mg/kg, with high variations due to species, variety, ripening stage, or local factors [6, 15].

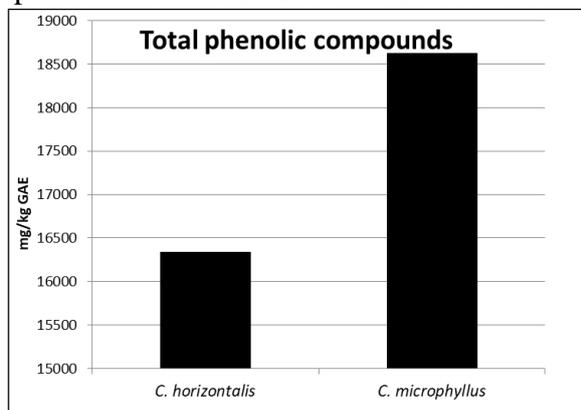
The total concentration of phenolic and polyphenolic compounds (including flavonoids) averaged 16342.06 mg/kg GAE (DW; 14983 - 17558 mg/kg) in *Cotoneaster horizontalis* and 18631.35 mg/kg GAE (DW; 18417 - 18846 mg/kg) in *Cotoneaster microphyllus* (Figure 5). These values are similar to those found in *Cotoneaster horizontalis* fruits (14000 mg/kg GAE) in a previous study [1] and lower than those in *Cotoneaster multiflorus* (38600 mg/kg GAE) [3].



**Figure 4.** Total flavonoids concentration in selected *Cotoneaster* species fruits (average values; mg/kg)

For comparison, the related *Pyracantha crenulata* species contains 7430 mg/kg total phenolics in fruits [15]. More than 10000 mg/kg were found in *Sorbus domestica* fruits [16], and 19150 mg/kg in *Sorbus torminalis* fruits [17]. A study on other domestic cultivars of *Sorbus* species found 362 - 8142 mg/kg GAE [6] while 2218 - 9843 mg/kg GAE were found in wild forms [18].

Total antioxidant capacity (TEAC) of fruits pulp hydroalcoholic extracts was reported according to the photochemiluminescence method, ACL procedure of Analytik Jena AG and quantified by comparison with Trolox® standard substance. Results are shown in Table 2.



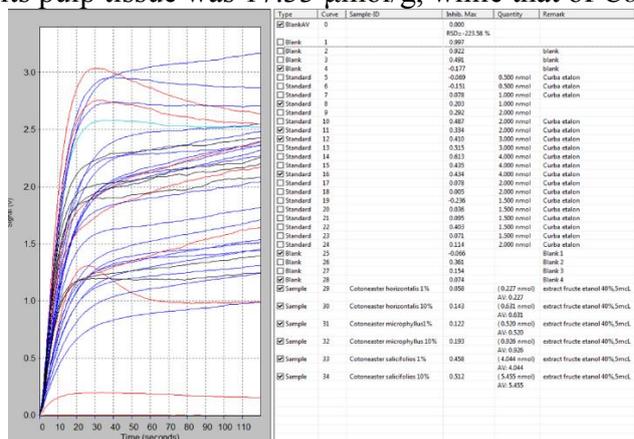
**Figure 5.** Concentration of total phenolic compounds in selected *Cotoneaster* species fruits (average values; mg/kg)

For both species, 10% extracts yielded significantly higher antioxidant capacities than 1% extracts (178% higher, for *Cotoneaster horizontalis* and 78% higher for *Cotoneaster microphyllus* pomes). However, when reported to initial fruits biomass, 1% extraction proved more efficient [23-30].

**Table 2**  
Total antioxidant capacity (TEAC) of *Cotoneaster* fruits hydroalcoholic extracts

No.	Sample Type	Extraction time (days)	Working volume (µL)	Analysis time (sec.)	Free radicals Inhibition Max.	Quantity means (TEAC) (µmol equiv. Trolox/g tissue)
1.	<i>Cotoneaster horizontalis</i> 1% in ethyl alcohol 40%	12	10	120	22.70	2.27 (17.33 DW)
2.	<i>Cotoneaster horizontalis</i> 10% in ethyl alcohol 40%	12	10	120	63.10	0.63 (4.81 DW)
3.	<i>Cotoneaster microphyllus</i> 1% in ethyl alcohol 40%	12	10	120	52.00	5.20 (39.69 DW)
4.	<i>Cotoneaster microphyllus</i> 10% in ethyl alcohol 40%	12	10	120	92.60	0.93 (7.10 DW)

Considering the initial dry biomass, the maximum antioxidant activity of *Cotoneaster horizontalis* fruits pulp tissue was 17.33 µmol/g, while that of *Cotoneaster microphyllus* was higher, 39.69 µmol/g.



**Figure 6.** ACL procedure curves for *Cotoneaster horizontalis* and *Cotoneaster microphyllus* fruit extracts samples



A close relative of *Cotoneasters* species is the medicinal plant *Pyracantha crenulata*. Its fruits were found to yield 13.00 - 32.98  $\mu\text{mol}$  Trolox equivalent/g antioxidant capacity [15].

Higher values were found in rowanberries (*Sorbus* species), 49.00 – 476.00  $\mu\text{mol}$  Trolox equivalent/g [6].

#### 4. Conclusions

Having average contents of 380.63 mg/kg, respectively, 179.63 mg/kg, *Cotoneaster horizontalis* Decne. and *Cotoneaster microphyllus* Wall. ex Lindl. species fruits were similar to related Rosaceae fruits, including *Sorbus* cultivars, and could be a valuable source of vitamin A and other important carotenoid-derived compounds.

With 8036.07 mg/kg, respectively 6888.06 mg/kg flavonoids content, they were also comparable to *Sorbus* species and other wild and cultivated Rosaceae. The same can be said for total phenolic and polyphenolic compounds content (16342.06 mg/kg GAE, respectively 18631.35 mg/kg GAE).

While the concentration of phenolic compounds (including flavonoids) was similar in the two species, the amount of carotenoids was significantly higher in *Cotoneaster horizontalis* pomes.

With up to 39.69  $\mu\text{mol}$  Trolox equivalent/g DW, the total antioxidant capacity of *Cotoneaster microphyllus* pomes is increased, and similar to that of some related medicinal and nutrition-use Rosaceae species.

Further research is needed to assess, in detail, the composition in bioactive compounds and to confirm the potential of these vegetal species for valuable applications other than ornamental ones.

**Acknowledgement:** This work was supported by a grant of the Romanian Ministry of Research and Innovation, CCCDI - UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0689 /P1. „*Lib2Life - Revitalizarea bibliotecilor si a patrimoniului cultural prin tehnologii avansate*” within PNCDI III

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Manuscript received: 28.08.2019