

## TOTAL PHENOLICS, FLAVONOID CONTENT AND ANTIOXIDANT POWER OF LEAF, FLOWER AND FRUITS FROM CORNELIAN CHERRY (*CORNUS MAS* L.)

M. S. STANKOVIC<sup>1</sup>, M. ZIA-UL-HAQ<sup>2</sup>, B. M. BOJOVIC<sup>1\*</sup> and M. D. TOPUZOVIC<sup>1</sup>

<sup>1</sup> University of Kragujevac, Department of Biology and Ecology, Faculty of Science, 34000 Kragujevac, Serbia

<sup>2</sup> The Patent Office, Karachi-75270, Pakistan

### Abstract

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This paper presents new data for comparative analysis of different plant parts (leaf, flower and fruit) of Cornelian cherry (*Cornus mas* L.). Total phenolic content, flavonoid concentrations and antioxidant activity are analyzed in fifteen different extracts from plant parts using *in vitro* standard spectrophotometric methods. The total phenolic content in the examined extracts range from 12.77 to 341.09 mg GA/g. The concentration of flavonoids in extracts is between 3.53 and 149.97 mg RU/g. Obtained values indicate that all plant parts of *C. mas* are very rich in phenolic compounds and flavonoids, especially. Antioxidant activity of extracts is determined using DPPH reagent and expressed as IC<sub>50</sub> values (mg/ml) that range from 518.47 to 11.06 µg/ml. Parallel to the analysis of *C. mas* plant parts, *Ginkgo biloba* standardized extract and three standard substances are analyzed for comparison. Ethyl acetate extract from fruit showed significant antioxidant activity, IC<sub>50</sub> values of this extract are higher than the values for the standard substance (chlorogenic acid) and *G. biloba* extract. New data regarding the flower extracts from *C. mas* shows a greater activity of the methanolic extract of flowers if compared to *G. biloba*. Acetone, as well as methanolic and water extracts from leaves show very strong activity. The report related to our research of plant parts of *C. mas* could help in the explanation of their uses in agriculture, food industry and pharmacy as well as can be regarded as promising candidates for natural plant sources of high antioxidant value.

**Key words:** *Cornus mas*, phenolic compound, natural antioxidants

### Introduction

European cornel or Cornelian cherry (*Cornus mas* L.) is a species of genus Dogwood (*Cornus* L.) and belongs to the family Cornaceae Link. It is a deciduous shrub or small tree up to 8 m high. The leaves are broadly elliptical, 5-8 cm long and up to 3 cm wide. Ten to twenty yellow flowers are arranged in an umbel inflorescence. The fruit is an oblong red drupe containing a seed. It inhabits rocky limestone areas, dry meadows and pastures, the edges of sparse oak forest up to 1300 meters above sea level in Europe and southwest Asia (Ball, 1968).

Plant parts of *C. mas* are very rich in phenolic compounds with very strong biological activity and are widely used in tra-

ditional and modern medicine and pharmacy, cookery and food industry. The fruit of this species is edible and is widely used in the preparation of juice, tea, jam and sweets. Fruit possesses astringent, anti-inflammatory, antioxidative and anthelmintic activity (Gulcin et al., 2005; Pantelidis et al., 2007; Paulovicsova et al., 2009; Yilmaz et al., 2009; Rop et al., 2010). Medicinally active substances in *C. mas* fruit are phenolic compounds, mineral substances, vitamins - especially vitamin C - and tannoid substances. Flavonoid compounds, especially the subclass of anthocyanins, are very numerous (Tural and Koca, 2008).

Free radicals are atoms or groups of atoms that have at least one unpaired electron, which makes them highly reac-

\* Corresponding author: biljanab@kg.ac.rs

tive. They are produced during the normal metabolism or are induced by UV radiation and different pollutants from the environment. Increased concentration of free radicals causes many pathological conditions in organism such as inflammatory, neurological, psychiatric diseases and carcinogenesis etc. (Chan, 1994; Salim, 1996; Valko et al., 2006).

Negative effects of free radicals can be largely prevented by intake of antioxidant substances. Antioxidants act by donating an electron to a free radical and converting it to a non-radical form (Ben-Shaul et al., 2000). Antioxidants can be of synthetic origin and a great number of secondary metabolites isolated from plants, such as various phenolic compounds. Natural antioxidants do not show a negative effect and allow unrestricted use. The most widely used synthetic antioxidants have been suspected to cause or promote negative health influences and genotoxic effects (Branen, 1975; Chen et al., 1992).

Plants are source of the most potent free radical scavengers such as different phenolic compounds and vitamins. Therefore, the investigations of biological activity and chemical composition of medicinal plants as a potential source of natural antioxidants are numerous in the recent studies (Panchawat et al., 2010; Denev et al., 2013).

The present study is prompted by the fact that no data on antioxidant activity, phenol concentration and flavonoid content of plant parts of *C. mas* has been provided so far, and in the literature there is no data concerning the comparative analysis of the antioxidant activity of different plant extracts from *C. mas*. The basic aim of the research was to determine the contents of phenolics and concentrations of flavonoids in various extracts (methanolic, water, ethyl acetate, acetone and petroleum ether from leaves, flowers and fruit) of the species *C. mas* using spectrophotometric methods, as well as to examine the antioxidant activity of plant extracts *in vitro* using standard model system. Also, we compare the obtained values of antioxidant activity with values of standard synthetic antioxidants and those related to extract from Ginkgo (*Ginkgo biloba*) as most popular plant rich in natural antioxidants.

## Material and Methods

### Chemicals

Acetone, methanol, petroleum ether, ethyl acetate and sodium hydrogen carbonate were purchased from "Zorka pharma" Šabac, Serbia. Standards of phenolic acids (gallic acid) and of flavonoids (rutin hydrate), chlorogenic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Chemicals Co., St Louis, MO, USA. The Folin-Ciocalteu's phenol reagent, 3-tert-butyl-4-hydroxyanisole (BHA) and

aluminium chloride hexahydrate were from Fluka Chemie AG, Buchs, Switzerland. A standardized extract of *Ginkgo biloba* was obtained from Pharmaceutical Company „Ivančić i Sinovi“, Belgrade, Serbia. All other solvents and chemicals were of analytical grade.

### Plant material

Flowers of *Cornus mas* L. were collected in March and leaves and fructus in August 2010 from the region of Pčinja river gorge in south Serbia. The voucher specimen of *C. mas* was confirmed and deposited in Herbarium at the Department of Biology and Ecology, Faculty of Science, University of Kragujevac. The collected flowers and leaves was air-dried in darkness at room temperature (20°C). Harvested fresh fruits are immediately used to prepare extracts.

### Preparation of plant extracts

The prepared plant material from *C. mas* (10 g) was broken in the small pieces 2-6 mm by using a cylindrical crusher and extracted with water and different organic solvents (methanol, acetone, ethyl acetate and petroleum ether), using Soxhlet apparatus. The extract was filtered through a filter paper (Whatman, No. 1) and evaporated. The residue was stored in a dark glass bottle for further processing.

### Determination of total phenolics in the plant extracts

Total soluble phenolic compound in the different extracts of *C. mas* were determined with Folin-Ciocalteu reagent using gallic acid as a standard (Singleton et al., 1999). Methanol extract was diluted to the concentration of 1 mg/ml and 0.5 ml of the soluted extract was mixed with 2.5 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and 2 ml of NaHCO<sub>3</sub> (7.5%). After 15 min of staying at the 45 °C, the absorbance was measured at 765 nm versus blank sample on spectrophotometer (ISKRA, MA9523-SPEKOL 211). Content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg GA/g extract). Values were uniformly expressed as the corresponding dry weight of plant extract (1 g). All measures were repeated three times.

### Determination of total flavonoids in the plant extracts

The total flavonoid contents were determined spectrophotometrically (Quettier et al., 2000). Briefly, 0.5 ml of 2% solution of AlCl<sub>3</sub> in methanol was mixed with the same volume of extract (1 mg/ml). Absorption readings at 415 nm were taken after 1 h against a blank (methanol). The total flavonoid content was determined using a standard curve with rutin (0-50 mg/L). Values were uniformly expressed as the corresponding dry weight of plant extract (1 g). All measures were repeated three times.

### Evaluation of antioxidant activity

The ability of the plant extract to scavenge DPPH free radicals was assessed by the standard method (Tekao et al., 1994), modified with suitable modifications (Kumarasamy et al., 2007). DPPH (20 mg) was dissolved in methanol (250 ml) to obtain a concentration of 80 µg/ml. The stock solution of plant extract was prepared in methanol to achieve the concentration of 1 mg/ml. Dilutions were made to obtain concentrations of 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.99, 0.97 µg/ml. Diluted solutions (1 ml each) were mixed with DPPH (1 ml). After 30 min in darkness at room temperature (23°C), the absorbance was recorded at 517 nm. The control samples contained all the reagents except the extract. The percentage inhibition was calculated using equation: % inhibition = 100 x (A control – A sample)/A control, whilst IC<sub>50</sub> values were estimated from the % inhibition versus concentration sigmoidal curve, using a non-linear regression analysis. The data were presented as mean values ± standard deviation (n = 3).

### Statistical analysis

All experimental measurements were carried out in triplicate and are expressed as average of three analyses ± standard deviation. The magnitude of correlation between variables was done using a SPSS (Chicago, IL) statistical software package (SPSS for Windows, ver. 17, 2008).

## Results and Discussion

### Total phenol content and flavonoid concentrations of the extracts

Fifteen leaf, flower and fruit extracts using water and four different organic solvents (methanol, acetone, ethyl acetate and petroleum ether) have been prepared to examine the free radical scavenging activity and contents of total phenols and flavonoid concentrations. The extraction solvents of different polarity are used to extract the active substances of different polarity.

The total phenolic content in the examined plant extracts using the Folin-Ciocalteu reagent is expressed in terms of gallic acid equivalent, GAE (the standard curve equation:  $y = 7.026x - 0.0191$ ,  $r^2 = 0.999$ ) as mg of GA/g of extract (Table 1). The concentrations of phenols in the examined extracts range from 12.77 to 341.09 mg GA/g. In methanolic, water and acetone extracts from leaves, very high values of total phenolic contents are measured. High concentration of phenols is in water (341.09 mg GA/g) extract.

In the analysis of results for the concentrations of total phenolic compounds in all leaf extracts, it is noticed that the highest concentration of phenolic compounds is in the extracts obtained using solvents of high and moderate polarity. The concentrations of phenols in the flower extract range from 42.04 to 187.94 mg GA/g. The highest concentration is in methanol and water extracts. In five different fruit extracts,

**Table 1**  
Total phenol content in the plant extracts expressed in terms of gallic acid equivalent (mg GA/g extr.)

Type of extract	Leaves	Flowers	Fruits
Methanol	192.21 ± 0.36	187.94 ± 1.49	31.36 ± 0.34
Water	341.09 ± 0.46	105.84 ± 0.92	12.77 ± 0.81
Ethyl acetate	65.13 ± 0.72	42.04 ± 0.62	179.05 ± 0.53
Acetone	239.97 ± 0.99	69.02 ± 0.77	55.38 ± 0.86
Petroleum ether	86.57 ± 1.04	31.52 ± 0.45	27.14 ± 0.33

Each value in the table was obtained by calculating the average of three analysis ± standard deviation

**Table 2**  
Total flavonoid concentrations in the plant extracts expressed in terms of rutin equivalent (mg Ru/g extr.)

Type of extract	Leaves	Flowers	Fruits
Methanol	41.64 ± 0.41	53.60 ± 0.84	7.18 ± 0.10
Water	22.18 ± 0.58	47.23 ± 0.30	3.53 ± 0.39
Ethyl acetate	149.97 ± 0.81	55.70 ± 0.21	41.49 ± 0.57
Acetone	115.00 ± 0.69	51.78 ± 0.55	8.05 ± 0.76
Petroleum ether	106.27 ± 0.52	49.65 ± 0.37	6.91 ± 0.09

Each value in the table was obtained by calculating the average of three analysis ± standard deviation

the highest content of phenols (179.05 mg GA/g) is measured in the ethyl acetate extract. In other extracts from fruit, the contents of phenolic compounds are low and vary from 12.77 to 55.38 mg GA/g. We conclude that the highest concentrations of phenolic compounds are extracted from fruit using moderate polar solvents.

All investigated plant parts of *C. mas* show high contents of phenolic compounds, but the leaves contain a large variety of phenolic compounds and their extraction depends on the polarity of the used solvent. Other authors also report that high dissolubility of phenols in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction (Zhou, 2004; Mohsen and Ammar, 2008). Using few extracts of different polarity has proved very effective for the purpose of quantification of phenolic compounds.

The concentration of flavonoids in plant extracts from leaves, flowers and fruit of *C. mas* is determined using spectrophotometric method with  $AlCl_3$ . The content of flavonoids is expressed in terms of rutin equivalent, RuE (the standard curve equation:  $y = 17.231x - 0.0591$ ,  $r^2 = 0.999$ ), mg of Ru/g of extract. The summary of quantities of flavonoids identified in the tested extracts is shown in Table 2. Values designating concentration of flavonoids in the extracts of leaves, flowers and fruit are very uneven. The concentrations of flavonoids in plant extract range from 3.53 to 149.97 mg RU/g.

The highest concentrations of flavonoids in leaves are measured in ethyl acetate, acetone and petroleum ether extracts, while methanolic and water extracts are with small amounts of flavonoids. Concentration of flavonoids in the extracts from flower is smaller in the comparison to extracts from leaves, but there is not much variation in the values. Their concentration values are from 47.23 to 55.70 mg RU/g. In extracts from fruit the highest concentration of flavonoids is in ethyl acetate extract, but other extracts from fruit do not exceed the value of 10 mg/g.

Based on the obtained values for concentration of flavonoids in the examined leaf extracts from *C. mas*, it has been found that the highest concentration of these compounds is in the extracts obtained using solvents of moderate polarity and non polar solvents. But, extraction of flavonoids from fruit is effective using only moderate polarity solvents such as ethyl acetate, while the effective extraction of flavonoids from flowers is achieved with all solvents. The results of analysis of fruit extracts indicate that ethyl acetate is the most effective solvent for extraction of phenolic compounds from the fruit of *C. mas*, and that moderately polar solvents should be used for this purpose.

The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation

(Min and Chun-Zhao, 2005). The total phenolic content and concentrations of flavonoids in the extracts of *C. mas* depend on the polarity of solvents and on the type of plant material used for extraction. Generally, higher concentrations of phenolic compounds are in the leaf extracts (Table 1).

#### Antioxidant activity

The antioxidant activity of plant extracts from *C. mas* is determined using methanol solution of DPPH reagent. DPPH method has also been used to quantify antioxidants in complex biological systems in recent years and based on the reduction of methanolic solution of colored free radical DPPH by free radical scavenger. The scavenging activity is measured as the decrease in absorbance of the samples versus DPPH standard solution.

The antioxidant activity of fifteen extracts from leaves, flowers and fruits of *C. mas* is expressed in terms of  $IC_{50}$  ( $\mu\text{g/ml}$ ) values (Table 3). Parallel to the examination of the antioxidant activity of the plant extracts, the values for three standard compounds and *G. biloba* extract (Table 4) - are obtained and compared to the values of the antioxidant activity. The standard substances used are rutin, chlorogenic acid and BHA.

The obtained values for antioxidant activity range from 518.47 to 11.06  $\mu\text{g/ml}$ . The largest capacity to neutralize DPPH radicals is measured in ethyl acetate extract from fruits of *C. mas*, which neutralized 50% of free radicals at small concentrations of 11.06  $\mu\text{g/ml}$ . Regarding total phenolic contents and flavonoid concentrations of fruit, ethyl acetate extract displays the highest values among all fruit extracts.

Concerning leaves extracts, acetone, methanolic and water extract show good activity (32.17, 39.40, 59.28  $\mu\text{g/ml}$  respectively), but none of them show higher activity than the ethyl acetate extract from fruits. These uneven values for the leaves extracts indicate that they contain compounds of different chemical structure and polarity. In the flower extracts, the highest antioxidant activity is measured in methanol (27.58  $\mu\text{g/ml}$ ) and water extract (36.78  $\mu\text{g/ml}$ ).

Very few researchers provide results of comparative analysis of leaf, flower and fruit of *C. mas*, mostly there are reports on the extracts from fruit of this species. Our data confirms the results of Serteser et al. (2009), who analyzed the antioxidant activity by three different antioxidant methods applied on leaves and fruit of *C. mas* and other plant species. In their results, higher antioxidant activity by all methods was found in *C. mas*. In comparative analysis of antioxidant activity of leaves and fruit performed by these authors, higher activity appeared at the extracts from fruit.

In comparison of  $IC_{50}$  values of BHA, rutin and chlorogenic acid (Table 4), ethyl acetate extract from fruit, wa-

ter extract from flowers and acetone, methanolic and water extract from leaves manifest strong capacity to neutralize DPPH radicals. Ethyl acetate extract from fruit, methanolic extract from flowers and acetone extract from leaves of *C. mas* are shown to be more active than *Ginkgo biloba* extract. Following a comparative review of six most active extracts of *C. mas* with values for standard substances, and *Ginkgo biloba* extract, the order of antioxidant power is BHA > rutin > ethyl acetate extract from fruit > chlorogenic acid > methanolic extract from flowers > acetone extract from leaves > *Ginkgo biloba* extract > water extract from flowers > methanol extract from leaves > water extract from leaves.

Comparative analysis of results for the concentration of phenolic compounds and antioxidant activity suggests that extracts with the highest concentrations of phenolic compounds also have strong antioxidant activity. This is confirmed by the statistical analysis of these values. Between the values of concentration of phenolic compounds (Table 1) and antioxidant activity of different plant extracts of *C. mas* (Table 3) a significant linear correlation has been proved (coefficient of correlation  $r = 0.717$ ). Obtained linear correlation demonstrates that the antioxidant properties are slightly better correlated to total phenolics.

Based on these results, all extracts of *C. mas* show a phenol concentration-dependent scavenging effect. Numerous investigations of the antioxidant activity of plant extracts have confirmed a high linear correlation between the values of phenol concentration and antioxidant activity (Moein and Moein, 2010).

In a detailed qualitative analysis of flavonoids in the extract from fruit of *C. mas*, eight compounds have been recognized as quercetin, kaempferol, and aromadendrin glycosylated derivatives (Pawlowska et al., 2010). For these three groups of flavonoids in the literature there are a large number of results to their strong antioxidant activity, especially in neutralization of DPPH radicals and other model systems (Teffo et al., 2009; Ahmeda et al., 2009). In our analysis of all the fruit extracts, ethyl acetate extract has the highest to-

tal phenolic content, highest flavonoid concentration and the strongest antioxidant activity; it is higher when compared to the activity of standard substance (chlorogenic acid) and *Ginkgo biloba*.

## Conclusion

The results of our study suggest the great value of the species *C. mas* for use in pharmacy and phytotherapy. Based on this, we conclude that the plant parts are natural sources of antioxidant substances of high importance. This contributes to its exploration, since there is no data in the literature as a comparative study of total phenol content, flavonoid concentrations and antioxidant activity of plant parts of *C. mas*. Further studies of this plant species should be directed to a detailed qualitative analysis of all its parts and carry out *in vivo* studies of its medicinal active components in order to prepare natural pharmaceutical products of high value.

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**Table 4**  
Values of antioxidant (DPPH scavenging) activity of standard substances obtained for comparison with *Cornus mas*

Substances	IC <sub>50</sub> µg/mL
BHA	5.39 ± 0.31
Rutin	9.28 ± 0.27
Chlorogenic acid	11.65 ± 0.52
<i>Ginkgo biloba</i>	33.91 ± 1.16

Each value in the table is obtained by calculating the average of three analysis ± standard deviation

**Table 3**  
Antioxidant (DPPH scavenging) activity of investigated plant extracts presented as IC<sub>50</sub> values (µg/ml)

Type of extract	Leaves	Flowers	Fruits
Methanol	39.40 ± 0.91	27.58 ± 0.64	251.87 ± 0.98
Water	59.28 ± 0.79	36.78 ± 0.48	518.47 ± 1.52
Ethyl acetate	381.34 ± 1.32	470.27 ± 1.21	11.06 ± 0.88
Acetone	32.17 ± 0.44	150.65 ± 0.95	107.99 ± 1.14
Petroleum ether	375.56 ± 1.01	390.21 ± 1.11	285.98 ± 1.69

Each value in the table is obtained by calculating the average of three analysis ± standard deviation

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