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Original Article

Antioxidant Capacity of Several Romanian Forest Fruits (*Rosa canina* L., *Prunus spinosa* L., *Vaccium vitis-idaea* L. and *Cornus mas* L.)

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Abstract

The comparison of the antioxidant activity of the studied forest fruits emphasized a hierarchy of the antioxidant capacity in rosehip, blackthorn, lingonberry and cornelian cherry. The purpose of the study was to investigate the antioxidant capacity and nutritional value of rosehip, blackthorn, lingonberry and cornelian cherry. In the current study, the FT-IR spectroscopy technique was applied to detect molecular components in forest fruits samples. Antioxidant capacity was evaluated with photochemical assay as well as humidity, protein, fibre, lipid and carbohydrate content. The FT-IR results revealed the presence of different bio-active compounds in berries such as flavonoids, tannins, sugars, acids, proanthocyanidins, carotenoids, citric metabolites and others. The highest antioxidant capacity was observed in rosehip 105.67±1.38 and blackthorn 49.89±1.92 (µg/mg equivalent ascorbic acid). Regarding nutritional parameters, rosehip showed the most increased content of protein displaying average values of 1.60, carbohydrates 38.20 and fibre 24.10 (g/100 g/sample). These results can provide useful information providing a research interest for the identification of new molecular compounds from Romanian flora samples.

Keywords: antioxidants; blackthorn; cornelian cherry; lingonberry; protein; rosehip

Introduction

Worldwide and from ancient times, traditional medicine played an important role, mainly for the treatment and prevention of various diseases, especially due to the use of active compounds derived from plants (Kinghorn *et al.*, 2011; Newman and Cragg, 2012; De Souza, 2014; Atanasov *et al.*, 2015). A large variety of biologically active substances such as anthocyanins, tannins and polyphenols, which can have a stimulating effect on the growth of probiotic microorganisms either deadly impact the life cycle of pathogenic bacteria, can be derived from plants (Scalbert, 2005; Meydani and Hasan, 2010; Khurana, 2013; Li *et al.*, 2014; Skrovankova *et al.*, 2015; Ozdal *et al.*, 2016).

Essential oils, plant extracts as well as fruit juices play an important role as raw materials in many products such as cosmetics, food industry constituents and medicine. Moreover, plant based natural products can be used as natural food additives instead of artificial food additives that are considered harmful to human health (Bridle and Timberlake, 1997; Laleh *et al.*, 2006). Furthermore the colourful fruits are considered a major source of antioxidant compounds such as phenolic pigments and anthocyanins that supply multiple beneficial health properties (Seeram *et al.*, 2001; He *et al.*, 2011).

The anthocyanins are a large subgroup of flavonoids, which are responsible for the production of bright colours from scarlet to blue or red to orange (Khoo *et al.*, 2017). The anthocyanins and the polyphenols derived from forest fruits/berries have recently drawn great attention due to their numerous health-promoting properties acting as free radical scavengers *in vivo* and *in vitro*, reducing considerably deleterious effects of free radicals which are mainly generated by reactive oxygen species (Tsuda *et al.*, 2000;

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Heo and Lee, 2005; Ercisli, 2007; Vergani *et al*., 2018; La Russa *et al*., 2019). The advantage of using phytochemicals is obvious due to many benefits as reduced toxicity, a wide range of pharmacological actions, rarely manifested side effects and infrequent presence of contraindications (Joseph *et al*., 2016; Umeno *et al*., 2016). The lingonberry or mountain cranberry (*Vaccinium vitis-idaea* L.) is a dwarf shrub from *Ericaceae* family, native to Europe, Scandinavian countries and Russia (Hokkanen *et al*., 2009). The entire plant contains a major source of procyanidins, polyphenols, anthocyanidins and flavonols, an abundant class of bioactive compounds found in nature (Bujor *et al*., 2018). The lingonberry phenolics include simple phenolic acids and flavonoids such as anthocyanins, proanthocyanidins, as well as flavonols that have been suggested to be responsible for many health benefits (Zushang, 2012). The fruits contain both A and B type procyanidins as well as a high concentration of oligomers and polymers of flavan-3-ols (Bujor *et al*., 2018). The procyanidins are known to have potent antioxidant capacities and may reduce the risk of chronic diseases, such as cardiovascular diseases and cancers (Prior and Gu, 2005; Schroeter *et al*., 2010). Type A procyanidins were suggested to contribute to lingonberries' preventative effects against urinary tract infections becoming the most popular dietary supplement used for the treatment of cystitis in worldwide (Foo *et al*., 2000; Ercisil, 2007; Kylli *et al*., 2011; Ştefănescu *et al*., 2019). The cornelian cherry (*Cornus mas* L.) is native in Asia and Europe as a slow-growing small tree or large shrub preferring sun or partial shade with well-drained soil. The fruits are rich in anthocyanin, flavonoids, polyphenol, vitamin C and acids such as ascorbic and loganic that exhibits powerful antioxidant properties and reduce significantly malondialdehyde levels and lipid peroxidation in experimental inflammation (Czerwińska and Melzig, 2018). The mature cornelian cherry fruits and its extracts have been used in traditional medicine for the treatment of gastrointestinal and metabolic disorders (Mamedov and Craker, 2004). Additionally, this fruits possess highly antimicrobial, anticarcinogenic and antidiabetic effects (Radovanović *et al*., 2013; Milenković-Andjelković *et al*., 2015; Dinda *et al*., 2016; Hosseinpour-Jsghdani *et al*., 2017).

Another European herb is rosehip (*Rosa canina* L.) from *Rosaceae* family and their fruits are considered an important source of nutrients (Tumbas *et al*., 2011). Aside from, the fruits contain high levels of vitamin C, carotenoids (lycopene, beta-carotene, rubixanthin) and polyunsaturated fatty acids (PUFA), which are compounds with elevated antioxidant activity (Angelov *et al*., 2014; Tumbas *et al*., 2011). The essential oils from rosehip seeds enclose significant levels of trans-retinoic acid with regenerative properties (Kiralan and Yildirim, 2019). Moreover, these oils exert antibacterial, and anti-inflammatory properties, and could also inhibit cancer cell proliferation (Olsson *et al*., 2004; Ayati *et al*., 2018). The seed of cornelian cherry and rosehip contain an important source of vitamins A, B1, B2 and K and minerals such as K, Ca, Na, Fe and Mg (Ozgan, 2002; Dzydzan *et al*., 2019).

The blackthorn (*Prunus spinosa* L.), an acid soils herb shrub from *Rosaceae* family, is native to Europe, Western Asia and Northwest Africa (Aliyazicioglu *et al*., 2015). The fruits have a rich content of polyphenols, sugars, vitamins, minerals, organic acids, beta-sitosterol, ferulic acid, anthocyanins, prunicyanine, gumiresines and tannins (Veličković *et al*., 2014; Balta *et al*., 2019). The fruits present diuretic, astringent, antidysentery and anti-diarrheal effects and they are recommended for gastric problems, kidney affections, dysentery, convulsive cough, diseases of the cardiovascular system and for the stimulation of digestion (Foito *et al*., 2018).

Global berries such as lingonberry, blackthorn, cornelian cherry, and rosehip are receiving particular attention for their significant content of phenolic compounds and vitamins, which can exhibit a wide range of pharmacological and biological properties such as antimicrobial, antidiabetic, anticancer, renal and hepatoprotective (Lares-Michel *et al*., 2019).

The fruits of *Cornus mas*, *Prunus spinosa*, *Rosa canina* and *Vaccinium vitis-idaea* selected in the current study, have a high content in phenolic substances and a high antioxidant capacity which can be used in food and pharmaceutical industries (Brown *et al*., 2012; Veličković *et al*., 2014; Dinda *et al*., 2016; Jimenez *et al*., 2017). The research interest regarding new sources of antioxidant and anti-inflammatory compounds based on natural products has a major increase. Moreover, experimental studies are necessary for assessments the biological activity of valuable forest fruits and to perform comparative quantifications of their curative properties.

Fourier transform infrared (FT-IR) spectroscopy is one of the most widely used methods to identify chemical compounds and elucidate the chemical structures because is a simple, rapid and accurate method to detect a chemical compound in food products and has a minimum sample preparation requirement (Sharma *et al*., 2017). This tool has recently been reported to be suitable for application in the pharmaceutical and food industry for the description of polysaccharide – polyphenolic conjugates and other secondary metabolites from plant-based extracts (Liu *et al*., 2018; Jafarirad *et al*., 2019; Opris *et al*., 2019).

Therefore, this work aimed to analyse and to achieve comparisons between molecular structures and antioxidant capacity of rosehip, cornelian cherry, lingonberry and blackthorn fruits dried at room temperature, using vibrational spectroscopic techniques (FT-IR) and in addition antioxidant capacity of lipid-soluble compounds was estimate.

Materials and Methods

Biological material

The samples were obtained from fresh lingonberry, rosehip, blackthorn and cornelian cherry fruits harvested at full maturity from four sites (1 - 46°40'54.6"N 23°05'46.1"E; 2 - 46°40'48.9"N 23°05'32.2"E; 3 - 46°40'32.1"N 23°05'48.3"E; and 4 - 46°41'19.3"N 23°05'27.2"E), located in Cluj County, Romania. The fruits were harvested early September and preventively washed with distilled water and then were used for further tests.

Moisture determination

The determination of moisture content was evaluated by the air-oven method, according to the methodology previously described by (Kumar and Balasubrahmanyam, 1986) with slightly modifications. The weighted sample was introduced into a vial containing lid and conditioned at 105 °C for at least 45 minutes until a constant mass was obtained and therefore cooled in air-oven. Then 5-10 g of sample is spread uniformly on the entire surface of the vial and weighted with an accuracy of 0.001 g. The humidity content was expressed in percentages.

FT-IR spectroscopy

The fruit samples (dried at room temperature) were obtained from 3 mg of each fruits used without further purification. FT-IR spectra were performed in the absorbance with a Jasco FT-IR-4100 spectrophotometer using KBr pellet technique.

The sample was prepared using calcinated potassium bromide as a matrix material and was mixed at a proportion of 3 mg of the sample to 200 mg KBr. Then the mixture was condensed in 15 mm die at a pressure equal to 10 t till 2 min. The same procedure was applied for the all samples (Crisan *et al.*, 2019)

Measurements were carried out on the infrared scale of 350-4000 cm^{-1} and a spectral resolution was set at 4 cm^{-1} and all spectra were acquired over 256 scans. The spectral data were analysed using Origin 6.0 software (Figs. 1-4). These spectra were analysed by comparing the obtained vibrational bands with those of similar functional groups from the literature.

Photochemical assay

The ACL (Antioxidant Capacity of the Liposoluble Compounds) method previously described by (Hegedus *et al.*, 2010), and slightly modified, was used for estimate the antioxidant capacity. Photochem® equipment (Analytik Jena AG, Jena, Germany) was used for measuring the antioxidant capacity. The equipment calibration and measurement of samples are based on the inhibition of free radicals. The amount of antioxidant capacity was achieved by establishing measurement curves that were compared to the measurement curves obtained for the standard solution. The calibration curve principle consists of determining the integrated calibration curve. Data was calculated automatically using a software program called PCL soft.

Statistical analysis

The IBM SPSS v.19.0 for Windows, was used for statistical analysis. Basic Statistics, was implemented in order to emphasize the arithmetic mean (X) ± standard deviations (SD) of the content of humidity and antioxidant capacity of the studied forest fruits.

The mean concentration of humidity and antioxidant capacity were compared across the various forest fruits using ANOVA followed by Tukey's test. Differences of the means were considered to be significant when $p\text{-value} < 0.05$.

Results

FT-IR analysis

The presence of flavonoids, pectin, proanthocyanidins, tannins, carotenoids, and sugars, fruit acids such as ascorbic acid, malic acid, and citric metabolites which possess antioxidant activity was reported, according to FT-IR analysis.

The analysis revealed that each particular polysaccharide has a specific band, with a maximum detected within 1200-1000 cm^{-1} region, which is assigned to stretching vibration of (C-OH) side groups, and the glycoside bond (C-O-C) vibrations in polysaccharide chains, as it can be observed in Figs. 1-4. In this regard the bands identified at 1079 cm^{-1} (blackthorn), 1077 cm^{-1} (cornelian cherry), and 1075 cm^{-1} (lingonberry) specific to this group are more intense in blackthorn, cornelian cherry and lingonberry, compared to rosehip (1079 cm^{-1}). This finding suggested that the rosehip have lower polysaccharide content.

The polyflavonoids showed an intense band at 3385 cm^{-1} presented in blackthorn, cornelian cherry and lingonberry. The bands with medium intensity identified at 3350 cm^{-1} were presented in the rosehip spectrum with a specific stretching vibration of O-H groups. The absorption bands for the carboxyl group and ester group may be found at 1720-1745 cm^{-1} (Pancerz *et al.*, 2019), and these groups are presented in all spectra having a high peak in rosehip.

The peak characteristics for asymmetric stretching vibration of-CH_2 corresponding to 2924 cm^{-1} (Santana *et al.*, 2016), and 2853 cm^{-1} which can be attributed to the stretching vibration of C-H, are noticeably increasing in rosehip fruits compared to other fruits were this peak was shifted (Fig. 2).

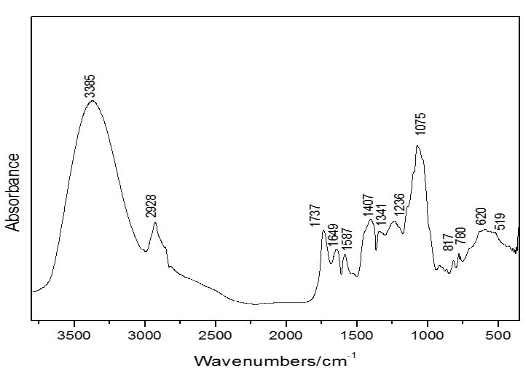

Fig. 1. FT-IR spectrum of lingonberry (*Vaccinium vitis-idaea*)

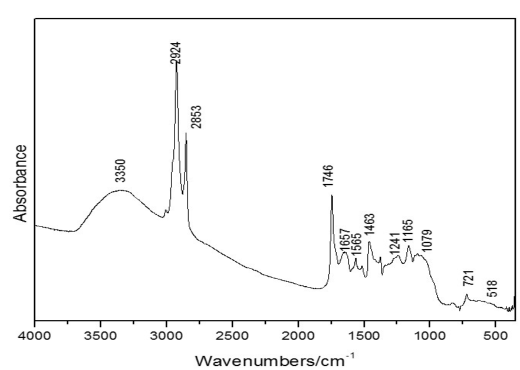

Fig. 2. FT-IR spectrum of rosehip (*Rosa canina*)

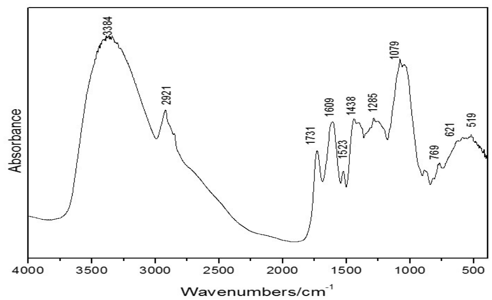

Fig. 3. FT-IR spectrum of blackthorn (*Prunus spinosa*)

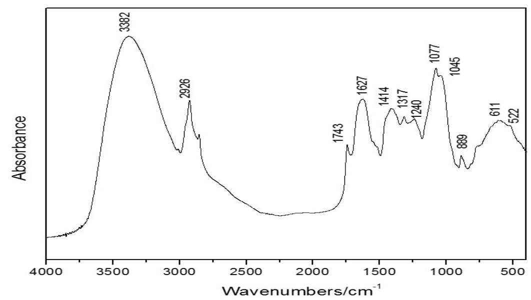

Fig. 4. FT-IR spectrum of cornelian cherry (*Cornus mas*)

Significant differences between all of the samples (Figs. 1, 2, 3, 4) were observed in the area 1750-1550 cm^{-1}, and this corresponds to the oscillation of the carbonyl C=O group of fructose and aldehyde CH=O of glucose (Saha *et al.*, 2007; Vardin *et al.*, 2008).

The 1627 cm^{-1} band is formed due to the stretching vibration of carbonyl group characteristic of the secondary amides and other compounds containing the C=0 group. The band at 1414 cm^{-1} represents the bending vibrations of CH indicative of the lignin and this band is more evidenced in cornelian cherry (Fig. 4).

The band located at 1241 cm^{-1}, corresponding to rosehip and cornelian cherry samples, seems to be linked with the presence of ester carbonyl groups. Another interesting region is typically for berries, and it is represented by bands in the range from 890-760 cm^{-1}, which corresponds to the specific oscillation of the anomeric region of carbohydrates, or C-H deformation. The region is described by considerable differences between particular samples, in a significant change of bond conformation (glycoside bond) (Samborska *et al.*, 2018). The absorbance of bands at 553-633 cm^{-1} indicates C-O-O and P-O-C bending of aromatic compounds such as phosphates.

Anthocyanin is an integrated molecule, which assure the transport of electrons through its structure (Cramer *et al.*, 2011). The absorption peaks of the forest fruits at around 519 cm^{-1} showed the presence of three anthocyanin pigments (Ramamurthy and Kannan, 2007).

The nutritional quality of the studied forest fruits

The nutritional values for 100 g of four types of forest fruits and the characteristic differences among them were present in Table 1.

The rosehip has the highest protein content, while the lowest protein content is reported in lingonberry. In addition, rosehips showed an elevated content of nutritional fibers and carbohydrates. In blackthorn was identified the highest concentration in lipid fraction. The fiber content identified in cornelian cherry was find bellow the limit of detection.

These differences reported in nutritional parameters of the studied forest fruits are the consequence of several reasons, the most relevant being: the species, the variety, and pedo-climatic conditions of culture.

Statistical analysis for the humidity content and antioxidant capacity

ANOVA analysis was followed by Tukey's test in order to perform multiple comparisons regarding the antioxidant capacity of considered forest fruits.

The Turkey test revealed significant differences between the mean amounts of antioxidants contained respectively in the four categories of forest fruits considered. The results of Turkey's test are displayed in Table 2.

The highest humidity content 74.30% was identified in cornelian cherry, while the lowest in cranberry, respectively 8.49%. Concerning the antioxidant capacity, the study showed that rosehip assert the highest value (105.67%), while the lowest values of 34.15% and 25.15% are reported for cranberry and cornelian cherry species .

Significant differences, at significance threshold of 0.05% are reported between the means of the antioxidant capacity of the studied forest fruits species, using Tukey test (Table 3).

Relationships among antioxidant capacity and humidity content of studied fresh forest fruits are emphasized using simple correlations. Significant positive correlations are reported between antioxidant capacity and humidity content in all the analysed samples: rosehip, blackthorn, cranberry, and cornelian cherry.

Table 1. Nutritional parameters of the investigated fruits (100 g/sample)

Name	Scientific name	Calories	Protein	Lipids	Carbohydrates	Fiber
Rosehip	*Rosa canina*	162 kcal	1.60 g	0.30 g	38.20 g	24.10 g
Blackthorn	*Prunus spinosa*	57 kcal	0.75 g	1.00 g	8.64 g	9.00 g
Lingonberry	*Vaccium vitis idaea*	46 kcal	0.46 g	0.13 g	12.00 g	3.60 g
Cornelian cherry	*Cornus mas*	76 kcal	0.70 g	0.40 g	16.90 g	0.00 g

Table 2. The content of humidity and antioxidant capacity of the studied forest fruits

Samples	Humidity (%)	Antioxidant capacity (µg/mg equivalent ascorbic acid)
Rosehip	30.30±1.17	105.67±1.38
Blackthorn	54.85±2.11	49.89 ±1.92
Cranberry	8.49±1.18	34.15±1.82
Cornelian cherry	74.30±2.10	25.15±1.65

Table 3. The differences and significance of differences concerning antioxidant capacity of the studied forest fruits (Tukey test)

	Rosehip	Blackthorn	Cranberry	Cornelian cherry
Rosehip	-	72.92*	93.42*	105.19*
Blackthorn		-	20.55*	32.35*
Cranberry			-	11.77*

Note: *The mean difference at significant $p < 0.05$.

Table 4. The correlation coefficients between antioxidant capacity and humidity content of the studied forest fruits

Antioxidant capacity (µg/mg equivalent ascorbic acid)	Rosehip	Blackthorn	Lingonberry	Cornelian cherry
Rosehip humidity (%)	0.98*	-	-	-
Blackthorn humidity (%)	-	0.91*	-	-
Cranberry humidity (%)	-	-	0.96*	-
Cornelian cherry humidity (%)	-	-	-	0.95*

Note: *Correlation is significant at $p < 0.05$.

A great correlation is identified between the mean humidity content in rosehip and antioxidant capacity, with a positive correlation coefficient of 0.98 (µg/mg equivalent ascorbic acid) while the lowest interaction, is identified in blackthorn, with a correlation coefficient of 0.91 (Table 4).

Conclusions

The resulted data revealed that the rosehip and blackthorn have the highest antioxidant capacity and the lowest antioxidant capacity was noticed in cornelian cherry. Moreover, the most increased nutritional indices were observed in rosehip and blackthorn. In conclusion, berries samples from Romania flora exhibit antioxidant capacity and more investigations should be performed on this topic to describe the complex interactions among antioxidants and the human body. It is promising to observe that molecular compounds, such as those in rosehip, blackthorn, lingonberry and cornelian cherry, may provide a potential interest in food and pharmaceutical research.

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Conflict of Interest

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

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