Antioxidant Activity, Total Phenol and Total Anthocyanin Contents of *Cornus sanguinea* L subsp *australis.* (C.A. Mey.) Jáv.

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Abstract

Background: ***Cornus sanguinea*** **L subsp** ***australis*** **(C.A. Mey.) Jáv. (Cornaceae) is a native species in north and northwest of Iran. It is locally named Siah-al. The genus** ***Cornus*** **is rich source of anthocyanins.**

Objective: In this study the antioxidant activity, total phenol and total anthocyanin contents of different extracts of ***C. sanguinea*** **L subsp** ***australis.*** **were investigated for the first time.**

Methods: The fruits were extracted with ethyl acetate, methanol (1% HCl) and water. DPPH and FRAP assay were performed for investigation of antioxidant activity of each extract. The total phenols were measured by Folin-Ciocalteu method while total anthocyanins were detected by spectroscopic method modified by Peksel.

Results: The results showed that ***C. sanguinea*** **L subsp** ***australis*** **methanol (1% HCl) extract (CME) had the highest amount of anthocyanins (12.56 ± 0.01 µmol/g extract) as well as the highest amount of total phenolics (88.56 ± 0.04 mg GAE/ g dry extract). The CME were found to have the highest antioxidant activity in DPPH assay (IC_{50}=90.43 µg/ml) and in FRAP method (1419.167± 0.025mmol FeII / g dry extract). Radical scavenger activity of CME at 100 µg/ml was comparable with α-tocopherol (20 µg/ml) and with BHA (200 µg/ml), p>0.05.**

Conclusion: There was a significant correlation between the total phenolic content an antioxidant activity of CME as well as total anthocyanin and antioxidant activity in DPPH assay (R^2 = 0.99). The results suggest that ***C. sanguinea*** **L subsp** ***australis*** **is a natural sources of anthocyanin and have considerable antioxidant activity.**

Keywords: ***Cornus sanguinea*** **L subsp** ***australis.*** **(C.A. Mey.) Jáv., Antioxidant, Total anthocyanin, Total phenol**

Introduction

The genus *Cornus,* known as dogwood, belongs to Cornaceae family and comprises about 55 species all over the world [1]. The most known species of this genus is *C. mas* (Cornelian cherry) which is widely distributed in Europe.

The leaves, flowers and fruits of *C. mas* has been used for more than 1000 years in traditional Caucasus and central Asian medicine to treat conditions like sore throats, digestion problems, measles, chickenpox, anemia, rickets, liver (hepatitis A) and kidney (pyelonephritis) diseases and diabetes [2]. The fruits of this species are also employed to treat circulation disorders, blood dilution and diabetes in Iranian traditional medicine [3]. It has also been used as food and cosmetic in Europe [1]. Other species of *Cornus* has also been reported to use in traditional medicine. For example, in traditional Chinese medicine *C. officinalis* has been used as tonic, analgesic and diuretic [1]. Fruits from *C. controversa* have been reported to use as an astringent and tonic in Korea and China. In addition, antimicrobial, antimalarial and antihistamine activity have been reported from this genus [1].

The *Cornus* plants are rich sources of bioactive substances such as phenolic compounds, mineral substances, vitamin C, tannoid compounds and anthocyanins [4].

So far, anthocyanins have been isolated from different species of this genus including *C. alternifolia*, *C. controversa*, *C. kousa*, *C. florida* [1], *C. officinalis, C. mas* [5] and *C. alba* [6]. In previous studies, 3-galactosides and 3-glucosides of pelargonidin, cyanidin and delphinidin have been isolated as the most relevant anthocyanins in *Cornus* spp [6]. Anthocyanins have been known for their wide range of health beneficial properties such as antioxidant, anti-inflammatory, anti-carcinogenic and proapoptotic activities. They also reduce the risk of cardiovascular disease and diabetes mellitus disorders [7].

In flora of Iran there are 2 species of *Cornus*: *Cornus mass* L. and *Cornus sanguinea* L subsp *australis* (C.A. Mey.) Jáv. The species *C. sanguinea* L subsp *australis* (C.A. Mey.) Jáv. is locally named Siyah-al and grown in north and northwest of Iran [8]. It is a shrub growing up to 5 meter high with black autumn berries. The literature survey shows that the biological activity of *C. sanguinea* L subsp *australis* (C.A. Mey.) Jáv. is not investigated. In this report *in vitro* antioxidant activity, total phenol and total anthocyanin contents of different extracts of fruits were investigated for the first time.

Material and Methods

Plant material

The fruits of the plant were collected from Vali-abad, Mazandaran province, Iran in October, 2012. A voucher specimen of plant (6751-TEH) was deposited in Herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Extraction

The fruits (1 Kg) were aired dried in shade and extracted with ethyl acetate, methanol (1%HCl) at room temperature, and water (40 °C), for 72 hours consequently. All the extracts were dried using rotary evaporator to give 120g, 198g and 63g residues from ethyl acetate, methanol (1% HCl), and water (40 °C), respectively.

Determination of total phenolic contents

Total phenolic contents of extracts were determined using Folin-Ciocalteu method as described by Miliauskas G, et al [9]. The extracts (1ml) were mixed with 5 ml of Folin-Ciocalteu reagent (previously diluted to tenfold with distilled water) and allowed to stand at room temperature for 10 min. A 4 ml sodium bicarbonate solution (75 g/l) was added to the mixture. After 30 min at room temperature, absorbance was measured at 765 nm using a UV spectrophotometer (Pharmacia Biotech). Total phenolics were quantified by calibration curve obtained from measuring the absorbance of a known concentration of gallic acid (GA) standard (20 - 200 mg/l). The concentrations are expressed as milligrams of gallic acid equivalents (GA) per g dry extract [10].

Determination of total anthocyanin

The anthocyanin content of methanol (1%HCL) and water extract of fruits were done by spectroscopic method modified by Peksel, 2008 [11]. Briefly, Absorbance was measured at 530 and 657 nm and indicated as A_{530} and A_{657}. The extinction coefficient of 31.6 M^{-1} cm^{-1} was used to convert the absorbance values into anthocyanin concentration. The concentration was calculated using the following equation: anthocyanin concentration (μmol/g) = $[(A_{530} - 0.33 \times A_{657})/31.6] \times$ [volume (ml)/weight (g)] [12].

Antioxidant activity

Evaluation of antioxidant activity by DPPH radical-scavenging assay:

The antioxidant activity of extracts were measured by the DPPH (2, 2'-diphenyl-1-picrylhydrazyl) free radical scavenging method based on an established method [13]. Sample solutions (1 ml) in methanol at different concentration were added to DPPH methanol solution (2 ml, 40 μg/ml). The mixtures were incubated at room temperature for 30 min and the absorbance was measured at 517 nm. Vitamin E and Butyl Hydroxyanisole (BHA) were used as standard controls. IC_{50} values denote the concentration of the test samples providing 50% radical scavenging were obtained from graph-plotted scavenging percentage against extract concentration.

Evaluation of antioxidant activity using FRAP assay:

The FRAP (ferric reducing antioxidant power) assay was performed according to the method described by Benzie and Strain [14]. Briefly, the FRAP reagent contained 5 ml of a (10 mmol/l) TPTZ (2, 4, 6- tripyridyl- s- triazine) solution in 40 mmol/l HCL plus 5 ml of (20 mmol/l) $FeCl_3$ and 50 ml of (0.3 mmol/l) Acetate buffer, pH 3.6 which was prepared freshly and warmed at 37°C. Aliquots of 50 μl extract were mixed with 1.5 ml FRAP reagent and after incubation at 37°C for 10 min, absorbance of reaction mixture at 593 nm was measured by spectrophotometer. For construction of calibration curve, five concentrations of $FeSO_4$ $7H_2O$ (125, 250, 500, 750, 1000 mmol/l) were used and the absorbencies were measured as for sample solution. The antioxidant activities were expressed as the concentration of antioxidants having a ferric reducing ability equivalent for 1 mmol/l $FeSO_4$ [10].

Results

In this study, the fruits of *C. sanguinea* L subsp *australis.* were extracted with ethyl acetate, methanol (HCl 1%) and water, consciously. The total phenol content was measured by Folin-Ciocalteau method. The total phenolic contents of extracts were ranged from 20.13 to 88.56 mg GAE / g dry extract. The high content of phenols is measured in *Cornus* methanol extract (CME) 88.56 mg GAE / g dry extract (Table 1). The high concentration of anthocyanin was determined in CME (12.56 µmol/g extract). The investigation of antioxidant activity of the extracts was performed by DPPH and FRAPS methods. The CME showed the highest antioxidant activity in DPPH assay (IC_{50}=90.43 µg/ml) and in FRAP method (1419.167 ± 0.025 mmol FeII / g dry extract). Radical scavenger activity of CME at 100 µg/ml was comparable with α-tocopherol (20 µg/ml) and with BHA (200 µg/ml), $p>0.05$. As shown in Table 2, There was a significant correlation (R^2=0.99) between the antioxidant activity in DPPH method and total phenolic contents, as well as antioxidant activity in DPPH method and anthocyanin contents of fruits extracts.

Table 1- Antioxidant activity, total phenolic and total anthocyanin content of different extracts of *C. sanguinea* L subsp *australis.*

	DPPH (IC50: mg/ml-1)	FRAP (mmol FeII / g dry extract)	Total phenol contents (mg GAE/ g dry extract)	Total anthocyanins (µmol/g extract)
CEE	762.3 ± 0.21	158.51 ± 0.0122	20.13 ± 0.02	-
CME	90.43	1419.167 ± 0.025	88.56 ± 0.04	12.56 ± 0.01
CWE	269.75	432.5 ± 0.063	74.91 ± 0.01	2.37 ± 0.06

Key to extracts employed: CEE: *Cornus* Ethyl acetate Extract, CME: *Cornus* Methanol Extract, CWE: *Cornus* Water Extract.

Table 2- Correlation between antioxidant activity with phenolic and anthocyanin contents.

Phenolic Content (mg GAE/g extract) & DPPH (IC_{50})	Phenolic Content (mg GAE/g extract) & FRAP Value (µmol Fe +2/ g extract)	Anthocyanin Contents (µmol/g extract) & FRAP Value (µmol Fe +2/ g extract)	Anthocyanin Contents (µmol/g extract) & DPPH (IC_{50})
y = -9.5816x + 960.55	y = 14.58x - 222.25	y = 99.313x + 175.81	y = -42.788x + 587.1
R^2 = 0.995	R^2 = 0.6342	R^2 = 0.9991	R^2 = 0.6737

Discussion

Anthocyanins are a class of components belongs to flavonoids family of compounds. There are known as water soluble pigments in berries like blueberries, cherries, raspberries, strawberries, black currants, purple grapes. Recent studies have demonstrate that anthocyanins can play significant biological roles including antioxidant, anti-inflammatory, antimicrobial and anti-carcinogenic activities [15]. The genus *Cornus* has been known as a source of anthocyanins [1, 4]. In Iranian traditional medicine, *C. mas* has been used for treating circulation disorders, blood dilution and diabetes.

In this study, the total phenolic contents, total anthocyanin contents and *in vitro* antioxidant activity of *C. sanguinea* L subsp *australis.* was investigated for the first time. The results showed that the methanol (HCl 1%) extract of fruits is a rich source of phenolic compounds (88.56 ± 0.04 µg GAE/g extract). The total phenol content of CME was higher than fruit methanol extract of *C. sanguinea* (34.19 ± 0.25 µg GAE/g extract) grown in central Serbia [4] and leave methanol extract of *C. mas* (0.248 ± 0.41 µg GAE/g extract) grown in Turkey [16].

The concentration of anthocyanin was 12.56 and 2.37 (µmol/g extract) in CME and CWE, respectively. Other studies showed that the most relevant anthocyanins in *Cornus* species are 3-galactosides and 3-glucosides of pelargonidin, cyanidin and delphinidin [6]. For example, in fruits of *C. mas* anthocyanin content ranged from 1.3, in yellow-coloured fruit, up to 223 mg cyanidin-3-glucoside equivalents 100 g^{-1} fresh weight [17]. So, the high content of anthocyanin in the fruits of *C. sanguinea* L subsp *australis* can be due to existence of mentioned components.

There was a significant Correlation (R^2=0.99) between the antioxidant activity in DPPH method and total phenolic contents (Table 2). The results showed that with the decrease of total phenolic and total anthocyanin contents, the antioxidant activity of the fruits extracts reduced in both DPPH and FRAP assay. There was also a significant Correlation (R^2=0.99) between the antioxidant activity in DPPH method and anthocyanin contents of fruits extracts. In other studies, the correlation between polyphenols and antioxidant activity in fruits of *C. mas* (R^2 = 0.97) has been reported [18].

Conclusion

The results suggest that the fruits of *C. sanguinea* L subsp *australis* can be consider as a source of antioxidant. Anthocyanin-rich fruits of this native plant can be the subject of further investigations for its beneficial properties as well as isolation of active components.

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