

## Antimicrobial and Antioxidant Evaluation of Fruit Extract from *Cornus mas* L.

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### Abstract

In this present study, water and methanol extracts of Cornelian cherry fruits (*Cornus mas* L.) were studied for evaluating of antioxidant and antimicrobial properties. The antioxidant properties were evaluated by determination of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability and lipid peroxidation inhibition activity. Also total phenolic contents of the extracts were detected by Folin method. The extracts of the fruits have antioxidant potential. The DPPH radical scavenging activity was higher in water extract (36.6 %) than the methanol extract (30.6 %). Unlike, methanol extract of fruits have more activity (18.9 %) than water extract (16.7 %), also with higher phenolic compound contents. Antimicrobial activities of above extracts were also tested against 93 clinical isolates of human pathogenic strains belonging to 5 bacteria (*Enterobacter aerogenes*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) and 5 yeast species (*Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*) by disk-diffusion method. The results showed cornelian cherries are potentially rich source of antimicrobial agents. The most effective antibacterial activity was expressed by methanol and water extract of cornelian cherry fruit against *S. aureus* with 25 mm inhibition zone and 0.156 mg/ml Minimum Inhibitory Concentration (MIC) value. Only methanol extracts of the fruit have antifungal activity against tested human pathogen clinic isolates.

### Keywords

Antimicrobial activity, Antioxidant activity, Cornelian cherry

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## 1. INTRODUCTION

Fruits and vegetables are healthy and functional foods that associated with a reduced risk of major chronic diseases such as cancer and cardiovascular disease [1]. These properties of fruits and vegetables have been attributed to the various antioxidant compounds [2]. There is a lot of evidence to show that free radicals cause oxidative damage to proteins, lipids and nucleic acids. In present, the natural history or etiology or of a number of diseases including cancer and cardiovascular disease. Free radicals are the most factors responsible from these diseases [3]. Therefore, antioxidants, which can neutralize free radicals, may be having the most importance in the prevention of these diseases [4].

Fruits and vegetables contain many different antioxidant components. Flavones, flavonones, isoflavones, anthocyanins, catechin, and isocatechin are some polyphenols that are frequently components of the human diet demonstrated have strong antioxidant activities [5, 6].

Medicinal plants are the greatest source of a variety of drugs. Especially, the antimicrobial activity of plant extracts has many applications, including pharmaceuticals, natural therapies and alternative medicine [7]. In fact, fruits and vegetables not only a primary food source also they have contain a variety of bioactive components, which might have potential beneficial health effects. Therefore, fruits and vegetables exhibit important potency against human bacterial and fungal pathogens [8].

Cornelian cherry (*Cornus mas* L.) are widely grown in different region of Turkey. It ranges from a shrub to a small tree which is reaching a height of up 7-8 m, with dark brown branches and greenish twigs. The leaves are opposite, 4–10 cm long and ovate to oblong. The flowers are 5–10 mm in diameter with yellow petals and produced in clusters of 10–25 together in the late winter. The fruit is drupe, oblong, red about 2 cm long and 1.5 cm in diameter containing a single seed [9].

In Turkey, cornelian cherries are not only used fresh but also consumed for producing jam, fruit marmalade syrup and several types of soft drinks [10]. Cherries are rich source of vitamins and minerals, including potassium,  $\alpha$ -tocopherol, ascorbic acid, biotin, riboflavin and a great source of fiber and contain a high amount of anthocyanin [11, 12]. Besides consumption as food, the plant leaves, barks and fruits are used as traditional medicine in Turkey. The plant have been used for asthma attack, coughs, bronchitis, diarrhea, diabetes, hemorrhoids, gastrointestinal disorders, healing wound and as a diuretic, wound healing, anti-inflammatory, urinary anti-inflammatory, and enhance immune system [9].

Cherries has been still under researched for their fruits are rich in vitamin, organic acid, fatty acid and tannins [10, 13] especially in recent years studies have focused on the antioxidant activity of this plant [14]. Although few groups reported the antimicrobial screening of the extracts of *C. mas*, no specific study on antifungal activity of this plant extract. Dinda and coworkers reported in their review that a systematic study could be useful to develop a new therapeutic drug from *C. mas* [9].

To the best of our knowledge there has been only one report in the available literature that explaining the antifungal activity of extracts against *Candida albicans*. The specific objectives of this study were (1) to determine the total phenolic contents, and antioxidant properties of cornelian cherries, (2) to investigate the antimicrobial properties on some human pathogenic clinical isolates, especially different *Candida* species.

## **2. MATERIALS AND METHODS**

### **2.1. Preparation of Extracts**

The cornelian cherry (*C. mas* L.) fruits were collected at their optimum maturity from Bayırbağ region, Erzincan, Turkey. The fresh fruit samples were transported to the laboratory and the seeds were removed manually. The fruits were extracted with methanol in a Soxhlet apparatus for 24 h. Then methanol was evaporated with a rotary evaporator. The water extracts were also prepared by adding boiling water to 20 g of material in a glass flask and incubated at room temperature for 2 hours on a rotating shaker (200 rpm). The mixture was filtered using Whatman (No.1) filter paper and then the filtrate was lyophilized. All the extracts were stored in freezer at  $-24^{\circ}\text{C}$  until use [15].

### **2.2. Test Microorganisms**

Antimicrobial activity tests were carried out against clinical isolates of 53 bacterial strains and 40 *Candida* strains. Microorganisms were provided by Department of Clinical Microbiology, Medicine Faculty, Erzurum. Microorganism species, isolation origins and numbers were given in Table 1.

### **2.3. Antimicrobial Activity**

#### **2.3.1. Disk diffusion assay**

The methanol and water extraction of fruits were dissolved with solvents (methanol and sterile distilled water). Final concentration was adjusted 30 mg/ml. Antimicrobial test were carried out by disc-diffusion method, using suspension containing  $10^8$  colony forming unit (CFU)/ml of bacteria and  $10^6$  CFU/ml of yeast spread on nutrient agar. The disc (6mm in diameter) were

impregnated with extracts and placed on the inoculated nutrient agar (NA). Negative controls were prepared using the same solvents (methanol and water). Positive controls were used as Ofloxacin for Gram-positive bacteria, Cefaperazone–sulbactam for Gram-negative bacteria and Amphotericin B for *Candida* spp. The inoculated plates were incubated at 37°C for 24 h for bacteria and at 35°C for 48 h for yeast. Then antimicrobial activity was evaluated by measuring the inhibition zone [15].

### 2.3.2. Minimal inhibition concentration (MIC)

The minimal inhibition concentration (MIC) values were determined for the microorganisms, which were found to be sensitive in disc-diffusion assay. MIC values of the extracts against microbial strains were determined based on a micro-well dilution method. The inoculations of microorganisms were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Firstly, the extracts dissolved in 10 % dimethyl sulfoxide were diluted to 10 mg/ml and then serial two fold dilutions were made in a concentration range (0.078–10 mg/ml) in a sterile test tube containing nutrient broth. The 96-well plates were prepared by dispensing into each well 95 µl nutrient broth (NB) and 5 l of the inoculums. A 100 µl of extracts initially prepared at the concentration of highest concentration was added the first well, then 100 µl from serial dilutions was transferred into other wells. The plates incubated for 24 h (for bacterial strains) and 48 h (for fungal strains). The MIC was defined as the lowest concentration of the extracts to inhibit the growth of microorganisms [15].

### 2.4. Antioxidant Activity

The antioxidant activity was determined by the thiocyanate method. Stock solutions of extracts were prepared at 2 mg/ml concentration. Stock solutions were mixed with 2.5 ml of 0.02 M linoleic acid emulsion [contains an equal weight of Tween-20 (Sigma) in pH 7.4 phosphate-buffered saline], and the final volume was adjusted to 5 ml with phosphate-buffered saline (0.02 M, pH 7.4) in a test tube and incubated in darkness at 40 °C. Final concentrations of the extracts were 100 µg/ml. Butylated hydroxytoluene (BHT) was used as positive control (100 µg/ml). The amount of peroxide was determined by measuring the absorbance at 500 nm after coloring with FeCl<sub>2</sub> and thiocyanate after 24 h incubation. Lower absorbance indicated higher antioxidant activity. Measurements of antioxidant activity were carried out for three sample replications, and the values are the average of three replicates. This activity was given as percent Lipid Peroxidation Inhibition and calculated with the equation

$$\text{Lipid peroxidation inhibition (\%)} = \left[ \frac{\text{ControlAbs} - \text{SampleAbs}}{\text{ControlAbs}} \right] \times 100$$

## 2.5. DPPH Radical-Scavenging Activity

At first, 0.5 mM DPPH radical solution in methanol was prepared, and then 1 ml of this solution was mixed with 3 ml of the sample solution. Final concentrations of extracts were 100 µg/ml. BHT was used as a positive control at the same concentration. After incubation for 30 min in the dark, the absorbance was measured at 517 nm. Decreasing the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity. This activity was given as percent DPPH radical scavenging, and calculated with the equation

$$Activity\% = \left[ \frac{ControlAbs - SampleAbs}{ControlAbs} \right] \times 100$$

Control solution contained 1 ml of DPPH solution mixed with 3 ml of ethanol. The measurements of DPPH radical scavenging activity were carried out for three sample replications, and the values are an average of three replicates [15].

## 2.6. Determination of Total Phenolic Compounds

Extract solution was transferred into a tube and then final volume was adjusted to 4 ml by addition of distilled water. Afterward, 0.25 ml of Folin-Ciocalteu Reactive was added into this mixture and after 3 min 0.75 ml of Na<sub>2</sub>CO<sub>3</sub> solution was added. Mixture was shaken on a shaker for 2 h at room temperature and then absorbance was measured at 760 nm. Amount of total phenolic compounds was carried out for three sample replications, and the values are an average of three replicates. Gallic acid was used as the standard for a calibration curve. The phenolic compound content was expressed as gallic acid equivalent using the following equation based on the calibration curve:

$$Y = 0.2582X$$

Where Y is the absorbance of the sample and X is the gallic acid the equivalent (µg/ml) [15].

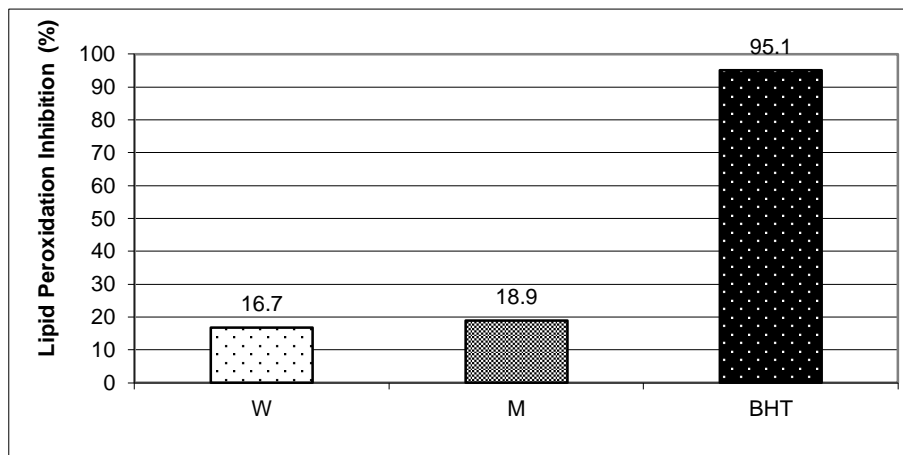
## 3. RESULTS AND DISCUSSION

### 3.1. Antioxidant Activities

The antioxidant activities of water and methanol extracts of Cornelian cherry fruits were determined using the thiocyanate method in which the amount of peroxides formed in linoleic acid emulsion during incubation is determined spectrophotometrically.

Although there was no too much difference between the antioxidant activities of water and methanol extracts, the high antioxidant activity was obtained from methanol extract, whereas the low activity was obtained from water extract cornelian cherry fruits (16.7 % and 18.9 %, respectively) (Figure 1).

The Cornelian cherry fruits are a rich source of high phenolic content, ascorbic acid and anthocyanin content, which are also responsible for color and taste and also their antioxidant properties [10, 16-18]. It was observed that all the extracts had antioxidant potential, and our findings were in agreement with these data.

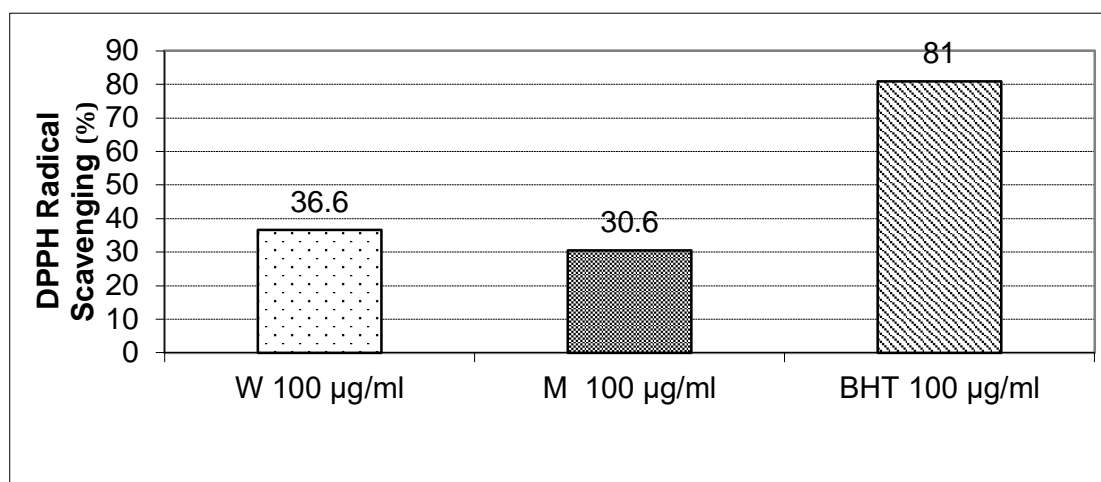


**Figure 1.** The Inhibition of Lipid Peroxidation by 100  $\mu\text{g/ml}$  extract and BHT. (M: Methanol extract, W: Water extract; BHT: Butylated hydroxytoluene)

The DPPH free radical has been used widely for determination of primary antioxidant activity, that is, the free radical scavenging activities of pure antioxidant compounds, plant and fruit extracts and food materials. The assay is based on the reduction of DPPH radicals in methanol and water, which causes an absorbance drop at 517 nm.

At the concentration of 100  $\mu\text{g/ml}$  water and methanol extract of cornelian cherry fruit, the scavenging activities were 36.6 % and 30.6 %, respectively (Figure 2). It is interesting to find that although the methanol extracts had the higher antioxidant activity; their DPPH radical scavenging activities were lower than the water extracts. However, when compared to the rates of DPPH activity of common synthetic antioxidants used in foods and fruit extracts, the DPPH radical scavenging activity of water and methanol extracts of cornelian cherry was lower than BHT (Fig. 2). The Cornelian cherry fruit juice was found to have strong antioxidant power, higher than that of other fruit juices [19]. Also, some authors were reported potential antioxidant activity of Cornelian cherry leaves and flowers [20, 21].

The total phenolic compounds present in the extracts were determined using the Folin-ciocalteu phenol reagent. Like Lipid Peroxidation activity, the amounts of total phenolic compounds were higher in the methanol fruit extracts than in the water extracts. The phenolic compounds content was present as 4.5  $\mu\text{g/ml}$  gallic acid equivalent in 100  $\mu\text{g/ml}$  methanol extract of cornelian cherry fruits, and the content was obtained from 100  $\mu\text{g/ml}$  water extract with 1.7  $\mu\text{g/ml}$  gallic acid equivalent (Table 1).



**Figure 2.** DPPH Radical Scavenging Activity, (M: Methanol extract, W: Water extract; BHT: Butylated hydroxytoluene)

**Table 1.** Total phenolic content of Cornelian cherry fruits

Fruit Extract	Gallic acid equivalent (µg/mL.)
Water	1.7
Methanol	4.5

Generally, the extracts that contain a high amount of phenolic compounds also exhibit high antioxidant activity [22]. Gil and coworkers reported that there was a strong correlation ( $r=0.930-0.960$ ) between total phenolic and antioxidant activity in some stone fruits [23]. This might be due to synergism among the antioxidants in the mixture. The cornelian fruits are rich in anthocyanins and phenolic contents with an average of 223–292 mg cyanidin 3-O-glucoside equivalents (CGE) and 281–704 mg Gallic acid equivalents (GAE) per 100 g of fresh fruits, respectively [17, 24]. Not only the fruits but also the leaves are rich in phenolic content (11.73%) compared to fruits (9.11%) [25].

### 3.2. Antimicrobial Activities

The antimicrobial activity of water and methanol extracts of cornelian cherry fruits evaluated by the disk-diffusion method. Totally 93 clinic isolates of human pathogenic microorganism belonging to 5 bacteria and 5 *Candida* spp. were used in these investigations (Table 2). The negative controls showed no inhibiting effect. The inhibition diameters and MIC values of positive controls were ranging to 18–20 mm; 0.12–1 µg/mL for Ofloxacin, 19–22 mm; 0.5–1 µg/mL for Cefaperazone and 12–15 mm; 0.12–0.5 µg/mL for Amphotericin B, respectively.

Our findings showed that the methanol and water extracts of fruits had antibacterial activity against Gram-positive and Gram-negative bacteria. The fruit extract proved to be active against 3 of the 5 bacteria species of human pathogenic clinic isolates. The highest antibacterial activity was expressed by methanol extract of cornelian cherry fruits against *S. aureus* with 25 mm

inhibition zone and 0.156 mg/ml MIC value (Table 3). Against *E. coli* and *P. aeruginosa*, 10 mm inhibition zone diameter and 0.312 mg/ml MIC values were found, respectively. *E. aerogenes* and *P. mirabilis* were not inhibited.

**Table 2.** Isolation origins and numbers of bacteria and *Candida* species

Microorganisms	Urine	Blood	Wound	Ear	Throat	Mouth	Total
<i>E. aerogenes</i>	8	-	2	-	-	-	10
<i>E. coli</i>	9	1	1	1	-	-	12
<i>P. miribalis</i>	7	1	1	1	-	-	10
<i>P. aeruginosa</i>	2	1	5	-	-	-	8
<i>S. aureus</i>	2	7	2	1	1	-	13
<i>C. albicans</i>	2	8	1	-	1	1	13
<i>C. glabrata</i>	5	-	-	-	2	-	8
<i>C. krusei</i>	-	1	2	-	1	-	4
<i>C. parapsilosis</i>	1	5	-	-	-	2	8
<i>C. tropicalis</i>	1	4	1	-	-	1	7
TOTAL							93

**Table 3.** Antimicrobial activity of methanol and water extracts of Cornelian cherry fruits against some clinic isolates.

Microorganism	Methanol Extracts	Water Extracts
	Inhibition Zone Diameter (mm) /MIC	mg/mL.
<i>E. aerogenes</i>	-	-
<i>E. coli</i>	10±1.6/(0.312)	10±1.3/(0.312)
<i>P.miribalis</i>	-	-
<i>P. aeruginosa</i>	10±1.2/ (0.312)	-
<i>S. aureus</i>	25±0.6/(0.156)	25±1.8 /(0.156)
<i>C. albicans</i>	8±0.3 /(0.625)	-
<i>C. glabrata</i>	-	-
<i>C. krusei</i>	-	-
<i>C. parapsilosis</i>	-	-
<i>C.tropicalis</i>	-	-

\_: Not active, inhibition zone was no greater than 6 mm; (7-12 mm), moderately active; (> 12), highly active

For screening antifungal activity of cornelian cherries, there was only one activity detected. Anticandidal activity was exhibited by methanol extract of fruits against *Candida albicans* with 8mm inhibition zone and 0.625 mg/mL MIC value. The fruit extracts exhibited no activity against 4 human pathogenic *Candida* spp. According to the results obtained in the course of current study, the *Candidal* strains were found to be resistant to both fruit extracts screened. Similarly, it has been previously reported that fungi are most resistant to plant extracts than bacteria [26].



Antimicrobial activity of leaf extracts of European cornel has been reported [27]. Hexane extract of Cornelian cherry seeds (10 mg/  $\mu$ L) showed significant antibacterial activity against *S. aureus* and *E. coli* [28]. On the other hand different authors [29] reported methanol and ethanol extracts of fruits, leaves and seeds of Cornelian cherry showed significant antibacterial activity against *S. aureus*, *E. coli* and *P. aeruginosa* and antifungal activity against *C. albicans* and *A. fumigates*. Also, Kyriakopoulos and Dinda mentioned fruit extract was exhibited strong antibacterial activity against *S. aureus* [30]. Our results are in agreement with these reports above that we also detected significant activity against *S. aureus* and moderate activity against *E. coli*, *P. aeruginosa* and *Candida albicans*.

## CONCLUSIONS

In conclusion, our findings on antibacterial activity of fruit extracts could justify some ethnopharmacological uses against diarrhea and gastrointestinal disorders because we detected activities of these fruits against some human pathogens (*E. coli*). On the other hand, the antioxidant activity could support the ethnopharmacological uses such as improving immune function. In addition, these results represent that these fruits are not only as a rich source of food but also as a valuable importance with high antioxidant and antimicrobial effects in ethnobotanical fields.

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