

Comparison among Cornelian Cherry and *Prunus cerasus* According to Phenolic Content and Antioxidant Capacity by Three Various Methods of Extraction

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Abstract

Cornelian cherry and *Prunus cerasus* with red pigments possess precious source of flavonoids and phenolic acids which have various applications in treatment of various health problems. This study is conducted to compare different methods of extraction (shaking incubator, soxhlet, ultrasonic) were applied in order to identify the best method which shows the highest rate of antioxidant capacity by DPPH and ferric reducing antioxidant power (FRAP) methods and total phenolic compounds via Folin-Ciocalteu procedure, p-coumaric acid content of fruits were evaluated by high performance liquid chromatography (HPLC). As a result, cornelian cherry with 1313.13 mg/Kg average TPC score exhibits higher total phenolic content than *Prunus cerasus* with 1270 mg/Kg. It's notice worthy that there was a slight difference among antioxidant activity in two fruits. Consequently, DPPH revealed nearly stronger antioxidant activity for *Prunus cerasus* while cornelian cherry had a little more potent antioxidant activity according to FRAP Test. p-coumaric acid content was almost twice in *Prunus cerasus* (10.8 mg/ml) than cornelian cherry (5.6 mg/ml). In addition, both shaking incubator and ultrasonic extraction procedures were more efficient than soxhlet in two fruits.

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Keywords

Cornelian Cherry, *Prunus cerasus*, p-Coumaric Acid, Total Phenolic Content, Antioxidant Activity

1. Introduction

Fruits with red pigments possess precious source of flavonoids and phenolic acids which have various applications in treatment of cancer and neurodegenerative diseases. Cornelian cherry (*Cornus mas*) is a species of flowering plant in dogwood family, is native to eastern Europe, Mediterranean countries (Italy, Spain) abundant in southern Belgium Luxemburg, central Germany, Middle eastern Asia (Turkey, Iran) central Asia, and south America [1]. *Cornus mas* is an excellent source of phenolic compounds (anthocyanin, flavonoids), antioxidants (butyl hydroquinone, butylated hydroxyanisole, and butylated hydroxytoluene) [2], high content of vitamins (E, B2, B1, and C), minerals (K^+ , Ca^{2+} , Mg^{2+} , Fe^{2+} , Zn^{2+} , Mn^{2+} , Cu^{2+} , Na^+) [3]. According to previous research, cornus juice is made up 10% tannins, 10% sugar, 5% pectin, 3% malic acids, 2% amberic acids, 1% ascorbic acids, glycosides, cartenoids, essential oils, and it has shown that the rate of Ascorbic acid in cherry is more than orange [1]. It's notice worthy that different parts of this plant (bark, leaves, and ripe fruit) have health benefits, for instance: all parts of plant have astringent properties [4], fruits are consumed to alleviate diarrhea and dysentery, sore throat, digestion problems, measles, chicken pox, anemia, diabetes [1]. In addition, flesh of fruit and seed oil is consumed for recovery and regeneration of damaged inner and outer epidemic tissues. A tincture that is made by the bark or the leaves of the plant can treat some health problems, for example: eczema, skin infection, intestinal parasites, veal skins, and gout [5]. Previous research has demonstrated cardio-tonic, anti-inflammatory and antioxidant, antidiabetic, anti-obesity activity of cornelian cherry [6] [7], it is proposed that different benefits of cornelian cherry are due to variety of phenolic acids, flavonoids, anthocyanin agents [8].

Prunus cerasus is a species of *Prunus* in the subgenus *Cerasus* (cherries) has different names: tart cherry, pie cherry, morello cherry, red cherry or sour cherry and is generated from hybridization between *Prunus avium* (sweet cherry) and *Prunus fruticos* (European dwarf cherry) in northern Iran and Turkmenistan, originated firstly from this place, has spread in Europe, and currently is cultivated in US [9]. Tart cherry is valuable source of vitamins (A, B1, B2, C, E, K, and Niacin), cartenoids like *beta*-carotene, minerals (Ca^{2+} , Fe^{2+} , K^+ , Na^+ , Mn^{2+} , and Phosphorous) [10], fiber, various suger like Fructose, Glucose, Maltose, antioxidant agents such as Caffeicacids, p-Coumaric acids, 1-(3',4'-dihydroxycinnamoyl)-cyclopenta-2,5-diol,1(3',4'-dihydroxycinnamoyl)-cyclopenta-2,3-diol, cyaniding-3-O-glucosylrutinoside [11] [12].

Prunus cerasus has some health benefits, including: decrease body weight and blood cholesterol of diabetic patients [13], antioxidant and anti-inflammatory effects [14]-[16], control the sleep-wake-cycle [10], reducing muscle pain [17] and prevent from symptoms of muscle damages [18].

The aim of this study is comparing different methods of extraction were applied for cornelian cherry and *Prunus cerasus* in order to compare extraction procedures and identify the best method which shows the highest rate of antioxidant activity and total phenolic compounds that save greater amount of beneficial agents in fruits. In addition, content of p-coumaric acid is evaluated by HPLC method in order to determine richer source of this beneficial agent among two precious fruits.

2. Material & Method

Chemicals: Gallic acid, Ascorbic acid, p-coumaric acid, DDPH (2, 2'-diphenyl-1-picrylhydrazyl), NaAC, $FeSO_4$ were purchased from *Sigma-Aldriche* company. TPTZ, $FeCl_3$, Acetic acid and Methanol 100% were prepared from *Merck* company in Germany.

2.1. Extraction Methods

Shaking Incubator: Firstly, fruit was bought from market and stored in $-20^\circ C$, 5 g of fruit was measured by measurement with accuracy 0.001 and crushed by mortar. Then, 50 ml methanol 100% was added and the mixture was poured into bottle and put it into shaking incubator with speed 150 rpm at $40^\circ C$ for 24 h. Finally, the extract was filtered.

Soxhelet Extractor: 5 g of fruit was measured and crushed, then it was packed into paper and put into soxhelet device. Candidate solvent was 50 ml methanol 100% and temperature was set on 80°C. After, the mixture was poured into rotator evaporator balloon and it was evaporated to 50 ml at 40°C with 75 rpm.

Ultrasonic Procedure: 5 g of fruit was measured, crushed and mixed with 50 ml methanol and put the mixture into ultrasonic device. The temperature was set at 40°C for 30 min then filtered.

2.2. Total Phenol Content

Total phenol method was applied according to Folin-Ciocalteu procedure. Standard for this assay is Gallic acid, 1mg/ml Gallic acid/distilled water was prepared, by rising the concentration of St from 10 to 60 µl, the color of complex would be from yellow to green and the rate of absorbance would be increased. For measuring TPC of fruit sample, five dilution (50, 70, 90, 110, 130 µl) of three types of fruit extraction method were analyzed, as an example for preparing aliquots (50 µl) fruit extract, (450 µl) distilled water and (500 µl) Folin-Ciocalteu were mixed and after 3 min, 500 µl Sodium Carbonate (0.1 N) was added to mixture, was placed 30 min in the dark at room temperature. Finally, the absorbance of prepared mixture against the blank at 765 nm was read by UV-Vis spectrophotometer. The results were expressed by g Gallic acid.

2.3. Antioxidant Activity

Antioxidant activity of sample were analyzed by applying two methods: DPPH, FRAP

DDPH (2, 2'-diphenyl-1-picrylhydrazyl) is a free radical scavenging assay that is on basis of transferring electron to produce free radicals. Hence, free radicals are reduced in the presence of antioxidant molecules because antioxidant agents act as H donor. Consequently, the rate of absorption in DPPH solution would be reduced by increasing the rate of antioxidant activity. 19.7 mg DPPH was measured and solute in methanol to reach 50 ml. The standard for this experiment is Ascorbic acid ;therefore, 17.5 mg Ascorbic acid/ distilled water was prepared, three dilution of standard (2, 5, 10 µl) were mixed with 350 ml DPPH and reached at 2 ml with methanol, calibration curve was drawn to achieve standard formulation. IC₅₀ values attribute to the concentration of the test samples providing 50% radical scavenging were obtained from graph-plotted scavenging percentage against extract concentration.

$$\% \text{ Inhibition} = \frac{A_{\text{DPPH}} - A_{\text{Sample}}}{A_{\text{DPPH}}} \times 100$$

For evaluating IC₅₀ of sample, five dilution of cornelian cherry extraction (10, 20, 30, 40, 50 µl) were applied then 350 ml DPPH was added, then whole mixture was reached to 2 ml by methanol, was placed in dark at room temperature for 30 min and observed by UV-Vis spectrophotometry. The absorbance of prepared mixture against the blank at 517 nm was read by UV-Vis spectrophotometer. The results were expressed by g Ascorbic acid.

2.4. FRAP

FRAP (Ferric reducing/antioxidant power) is a simple assay that estimates antioxidant capacity of supplements containing polyphenols. For preparing FRAP solution, three different solute should be made. 1) 146 µl HCL was added to 100 ml distilled water, then 93 mg TPTZ was added to 30 ml of this solute. 2) 162 mg FeCl₃ was added to 30 ml distilled water. 3) 930 mg NaAC was added to 4.8 ml Acetic acid, and reached to 300 ml by distilled water. It is notice worthy that PH of solution must be set at 3.6 by HCl 37%. As a result, FRAP solute is composed of 300 ml Acetic acid solute, 30 ml TPTZ, 30 ml FeCl₃ solute, 36 ml distilled water. Therefore, below reaction would be happened in FRAP solution that can be visualized at 593 nm by UV-Vis spectrophotometry:



The standard for this experiment is FeSO₄; therefore, 0.0139 g FeSO₄ as standard (st) was measured and mixed with 50 ml distilled water, five dilution of st (10, 20, 30, 40, and 50 µl) mixed with 1300 µl FRAP solution and mixture was reached at 1500 µl final volume by distilled water, calibration curve was drawn to achieve standard formulation. Then, three dilution of fruit sample (5, 10, and 15 µl) were added to 1300 µl FRAP solution, then mixture was reached at 1500 µl final volume by distilled water, and stored at room temperature in dark for 30 min, the absorbance of prepared mixture against the blank at 593 nm was read by UV-Vis spectro-

photometer. The results were expressed by g FeSO₄.

2.5. HPLC

In this experiment, 5 g of fruit sample was measured, crushed. 0.08 g Ascorbic acid was dissolved in 5 ml water, then crushed fruit and mixed with 25 ml methanol. Next, 3.5 ml HCL 37% dissolved in 30 ml water to achieve HCL 1.2 mol. The solution was put on water bath for 16 h at 35°C. After staying in room temperature, the solution was filtered and evaporated to dryness by evaporator rotary at 35°C. It's notice worthy due to existence of some oil in extract, the residue will be one drop of oil after dryness. The residue dissolved in 2 ml methanol and filtered by Whatman® GD/X syringe filters with pore size 0.45 µm, diam 13 mm for HPLC injection. The HPLC that exploited for this research was equipped with Agilent 1200 series (Agilent technologies Walbronn, Germany). It is consisted of a G1312B binary pump, a G1376A capillary pump, G1330B FC/ALS, G1379B Degasser, and G1377A microwips, controlled by Chemstation software. Chromatographic separations were conducted on columns with 4.6 × 150 mm, 5 µm ZORBAX Eclipse XDB C18 column (Agilent technology, Germany). Standard and extracts were run by two mobile phases: A) 0.1% phosphoric acid, B) (Methanol HPLC 100%). 10 µl standard (1 ppm) and extract should be injected into HPLC. The peak p-coumaric acid was revealed at 320 nm UV-Vis spectra.

3. Results

According to **Table 1**, two fruits with three various methods of extraction exhibited the rate of total phenol from at least 870 ± 64 to 1650 ± 330 mg/kg. By comparing the content of phenolics on basis of fruits, it is deduced that cornelian cherry with higher average TPC has almost higher total phenolic compounds than *Prunus cerasus*. In relation to extraction methods, shaking incubator and ultrasonic both revealed higher rate of total phenol than soxhelet in two fruits.

Second and third columns are related to antioxidant activity of fruits (DPPH, FRAP). There is a negative relationship among the score of DPPH score and antioxidant activity. Thus, by comparing two fruits, it is deduced that *Prunus cerasus* with 6.2 mg/ml DPPH total average score has almost stronger antioxidant activity than cornelian cherry. Likewise TPC evaluation, shaking incubator and ultrasonic are more efficient approaches for antioxidant activity in regard to their lower IC₅₀ score than soxhelet.

On the basis of FRAP's results, the antioxidant activity of three methods weren't so much different in cornelian cherry; However, ultrasonic method exhibited higher FRAP score than other methods and according averages concluded that cornelian cherry has stronger antioxidant activity than *Prunus cerasus* that isn't in accord of DPPH result.

HPLC: p-coumaric acid was distinguished by exploiting HPLC procedure to determine quantity of this agent in two fruits. p-coumaric acid was detected at Ret time 17.985, 320 nm HPLC running and the graphs are shown in **Figure 1** and the concentration of agent in fruits are exhibited in **Table 2**. According to **Table 2**, average agent concentration was about 5.6 mg/kg and 10.8 mg/kg in cornelian cherry and *Prunus cerasus*, respectively.

Table 1. Concentration of total phenols (TP) [mg GAE kg⁻¹], and antioxidant activity of fruits by two methods DPPH [mg·ml⁻¹], FRAP [µmol·g⁻¹], and three various methods of extraction. Values are means ± SD (n = 4).

<i>Cornelian cherry</i>	Σ Polyphenols mg/kg	DPPH (IC ₅₀) mg/ml	FRAP µmol/g
Shaking incubator	1650 ± 330	3.95 ± 0.18	190 ± 20
Soxhelet	870 ± 64	9.67 ± 2.8	200 ± 50
Ultrasonic	1420 ± 119	6.43 ± 0.34	190 ± 20
Total average	1313.3	6.68	193.3
<i>Prunus cerasus</i>			
Shaking incubator	1260 ± 310	5.85 ± 1.18	170 ± 20
Soxhelet	1080 ± 180	8.13 ± 0.89	170 ± 40
Ultrasonic	1470 ± 70	4.70 ± 1.23	200 ± 20
Total average	1270	6.2	180

Table 2. Concentration of p-Coumaric acid in cornelian cherry and *Prunus cerasus* ($\text{mg}\cdot\text{kg}^{-1}$ of fresh weight) determined by HPLC method and concentration of agent is higher in *Prunus cerasus*. Values are means \pm SD (n = 3).

Fruits	p-Coumaric acids concentration mg/kg
Cornelian cherry	5.6
<i>Prunus cerasus</i>	10.8

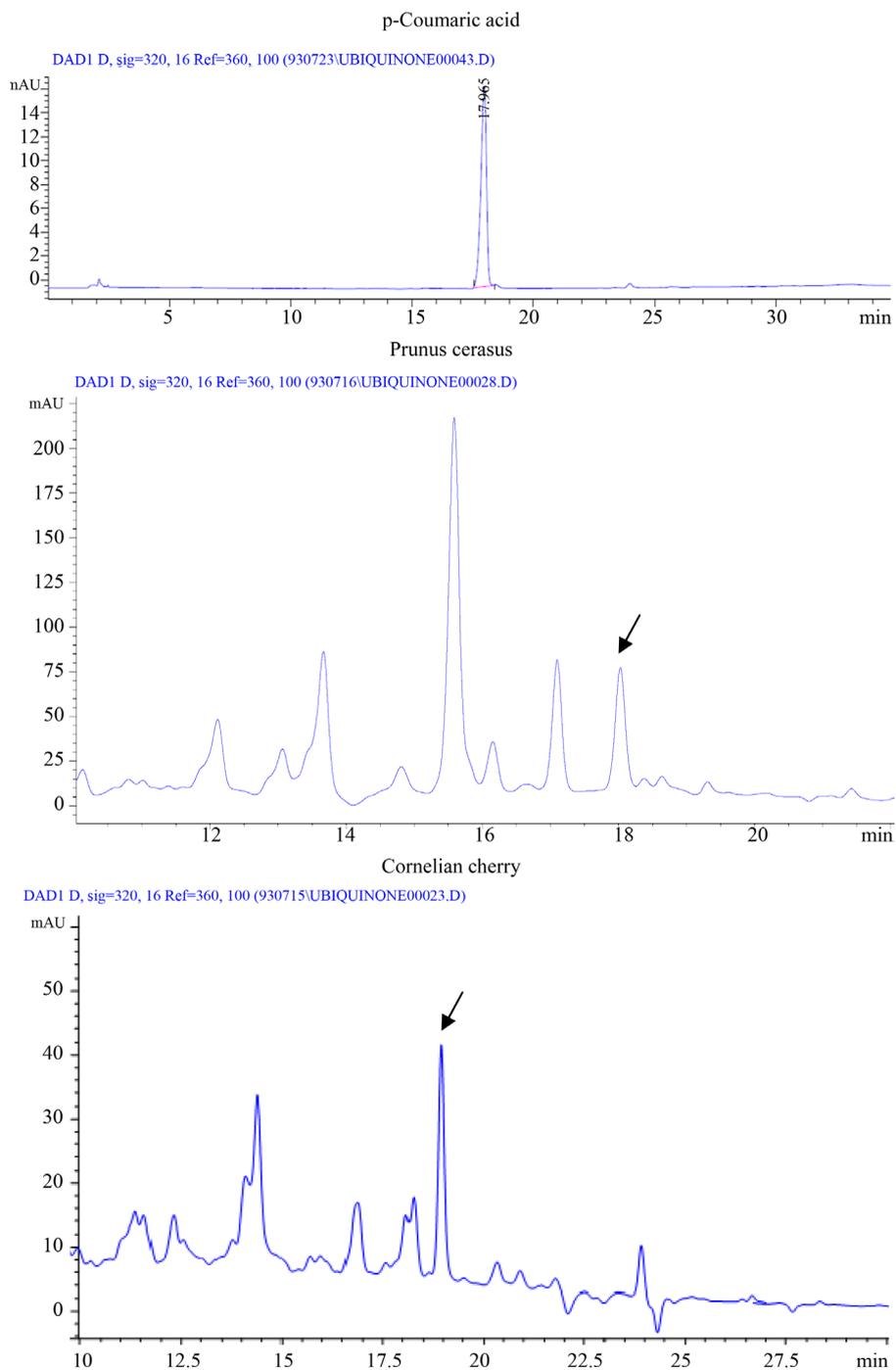


Figure 1. HPLC chromatograph of p-coumaric acid, *Prunus cerasus* and cornelian cherry that were detected at ret time 17.985 min, 320 nm.

Indeed, the content of p-coumaric acid is higher in *Prunus cerasus*.

4. Discussion

Total phenolic content score is versatile on basis of fruit type, stage of growth, farm of landing, extraction method, component of TPC experiment and other factors. Therefore, by comparing light yellow blush, light red and dark red of cornelian fruit total phenol content researchers found that by transforming fruit from first stages of growth to fully ripe form, the content of total phenol has decreased. Thus, dark fruit with 4162 mg/kg TPC had the lowest phenolic content, light yellow fruit with 8033 mg/kg was the richest phenolic compound source [19]. In regard to our study, the content of phenolics are about 1313.13 mg/kg that is almost 3 times less than reported TPC. In addition, another research conducted in Turkey to evaluate cornelian cherry properties, TPC reported for cornelian cherry was about 2810 mg/kg that was twice our reported TPC [8]. Another research did a comparison among partially and fully ripetart cherry fruit and concluded that by increasing the ripeness, the distribution of phenolic compounds will be increased. Consequently, fully ripe fruit with 3110 ± 44 mg/kg has higher TPC than partially ripe fruit (2170 ± 50 mg/kg) [20]. Although the tart cherry was applied in our study was fully ripened, the content of TPC was lower than reported TPC for ripefruit. Another study compared TPC of sweet and sour cherry and they found about TPC for sour cherry has spectra from 1461 to 3124 mg GAL/g that is compatible with our results [21].

DPPH activity of cornelian cherry was variant in our research on basis of the method and had spectra from 3.95 ± 0.16 to 9.67 ± 2.8 mg/ml that soxhelet because of exerting heating might have reduced the antioxidant activity. It's interesting that situation of cultivating has impression on antioxidant activity. Therefore, previous researchers by comparing DPPH activity of 12 cornelian cherry that farmed by various cultivars achieved DPPH score from 3.30 ± 0.20 to 9.54 ± 0.32 mg/ml and our finding is included in this spectra [22]. DPPH activity of various types of cherries are different. Melicháčová, S et al. have investigated total antioxidant activity of tart cherry and sweet cherry, they found that tart cherries possess 5.4 to 9.9 mg/ml DPPH inhibition potency. It's noteworthy that IC_{50} score was achieved in our study had spectra from 4.70 ± 123 to 8.13 ± 8.9 mg/ml that is consistent with reported IC_{50} [23].

Simonian, S conducted research about FRAP activity of cornelian cherry, and they reported 235 μ mol/g FRAP activity for this fruit that is in accord of our result [24]. In this experiment FRAP activity have spectra from 180 to 190 ± 40 μ mol/g, that is partially consistent with previous research. In addition, Ferric reducing ability of tart cherry has investigated by previous researcher and a number of pacific Northwest sour cherries in relation to FRAP activity was compared, it was found that *Prenus cerasus* with score 182.8 ± 0.45 μ mol/g can reduce ferric, this result is in accord of our result with spectra from 170 to 200 μ mol/g FRAP activity [25].

By comparing extraction approaches, it can be deduced that for analyzing TPC, shaking incubator and ultrasonic with higher score of TPC are more efficient methods than soxhelet and. It's notice worthy that although $40^{\circ}C$ is applied for 24 h in shaking incubator approach, the heat that is increased to $80^{\circ}C$ during soxhelet for about 2 h in this procedure may degrade more phenolics and the efficiency might be decreased. Moreover, for analyzing DPPH, shaking incubator extract with the lowest rate of DPPH score could save the antioxidant activity of fruits more than other methods. However, soxhelet with highest rate of DPPH in fruits is the least efficient method for analyzing antioxidant activity. While there wasn't so much difference in FRAP score with different extraction methods in cornelian cherry, FRAP score that was achieved by ultrasonic procedure was the highest in *Prunus cerasus*.

5. Conclusion

To sum up, between three methods of extraction shaking incubator and ultrasonic procedure were more efficient for evaluating total phenolic compounds and antioxidant activity. Moreover, by applying two methods of antioxidant activity analysis, it is concluded that there isn't considerable difference among cornelian cherry and tart cherry antioxidant activity due to fact that by DPPH test tart cherry exhibited stronger antioxidant activity, but cornelian cherry had more potent antioxidant activity via FRAP test .It's notice worthy that although p-Coumaric acid content also is higher in tart cherry, total phenolic content is slightly higher in cornelian cherry.

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