



## The effect of different solvents and ultrasound on antioxidant properties of extract of *Cornus mas* L. fruit

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**Introduction:** Cornelian cherry (*Cornus mas* L.), which belongs to the family Cornaceae, grows in Iran, in areas such as Qazvin and Arasbaran. The fruit possesses anti-inflammatory and antioxidant properties and it is used as an herbal remedy in medicine. Separation of natural antioxidant compounds from plant sources requires an appropriate method of extraction, which is effective factor to achieve the higher efficiency of these valuable compounds. In this study, the effect of extraction methods (immersion and ultrasound) and different solvents (ethanol 100%, ethanol – water (50:50 V/V) and water) on amount of phenolic compounds and antioxidant properties of cornelian cherry fruit extract were investigated.

**Materials and Methods:** Qazvin cornelian cherry was purchased from the local market of Amol city, Mazandaran province, Iran. All solvents and chemicals used in this study were of analytical reagent grade and were prepared from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO). Cornelian cherry was washed, core separated, dried in front of the sun for 5 days and then powdered with kitchen miller. Powdered cornelian cherry fruit was extracted using immersion extraction techniques, ultrasound and different solvents (ethanol 100%, ethanol –water (50:50 V/V) and water). In the immersion method, powdered cornelian cherry fruit were mixed with each solvent in the ratio of 1:10, individually. Then, the mixtures were shaken overnight at room temperature. After 24 hrs, the extracts were filtered through Whatman No. 42 filter paper and the solvents were evaporated in an oven at 55°C. In the ultrasound technique, the mixture of powdered samples with any solvent (1:10) was sonicated in an ultrasonic bath for 45 min at 35°C. The extracts were then filtered and the solvents were evaporated using an oven at 55°C. Finally, the extracts obtained from extraction methods were kept in a freezer for further experiments. The total phenolic content of the extracts was determined with the Folin-ciocalteu method, briefly, 0.5 mL of cornelian cherry fruit extracts with concentration of 1mg/mL were mixed with 2.5 mL of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and 2 mL of 7.5% sodium carbonate solution, then the samples were kept for 30 min at room temperature in the dark and at the end the absorbance of the solutions was read at 760 nm. The ability of the extracts to scavenge 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) was determined. 0.3 mL of each extract with a different concentration (500-3000µg/mL) was mixed with 2.7 mL of methanolic solution of DPPH ( $6 \times 10^{-5}$  mole/L), then the mixture was shaken vigorously and was placed in the dark for 60 min. Absorbance was recorded at 517 nm. The percentage of the DPPH radical scavenging was calculated according to the following equation:

$$\% \text{ inhibition of DPPH radical} = [(A_{\text{DPPH}} - A_s) / A_{\text{DPPH}}] \times 100$$

$A_s$  and  $A_{\text{DPPH}}$  are the absorbance of the solution the absorbance of the DPPH solution, respectively. Reducing power of extracts on iron ion was measured. 1mL of each extract with a different concentration (500-3000µg/mL) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH= 6.6) and 2.5 ml potassium ferricyanide [ $\text{K}_3\text{Fe}(\text{CN})_6$ ] (1%), then the mixture was incubated at 50° C for 30 min. After that, 2.5 ml of 10% trichloroacetic acid were added to the mixture, then, was centrifuged at 1000g for 10 min. Subsequently, 2.5 ml of the upper layer solution was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1%  $\text{FeCl}_3$ . Finally, the absorbance values of the solutions were read at 700 nm.

**Results and discussion:** The result of this study showed that the type of solvent and extraction method has been effective on amount of phenolic compounds of extracts, and also concentration dependent of phenolic

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compounds with antioxidant activity was observed in all extracts. The highest amount of phenolic compounds with 142.72 mg/g (based on Galic acid) was observed in sample extract obtained from solvent of water- ethanol (50:50 V/V) employing ultrasound method. Also, this extract with the lowest IC<sub>50</sub> value with the amount of 0.955 mg/ml in the DPPH free radical scavenging method and the highest absorption with the amount of 0.601 in the reducing power of Iron III test, the highest antioxidant performance is shown. A negative correlation was observed between the total phenolic content and the IC<sub>50</sub> value in the methods of measuring the antioxidant activity (DPPH and reducing power), which revealed the higher total phenolic content will give the lower IC<sub>50</sub>, that means the higher antioxidant activity. The results of present research showed that cornelian cherry fruit is a natural source of phenolic compounds and have considerable antioxidant activity.

**Key words:** *Cornus mas* L. fruit, Immersion, Ultrasound, Solvent, Antioxidant properties.