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Evaluation of antioxidant capacity of ethanol, acetone, ether and aqueous extract of mistletoe leaves

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Abstract

Mistletoe plant is one of the medicinal plants used to treat cancer, high blood pressure, fatigue and anxiety, inflammatory diseases and regulate the immune system. The aim of this study was to evaluate the antioxidant capacity of mistletoe leaf extract in aqueous, ethanol, ether and acetone solvents. In this laboratory experimental study, mistletoe plant leaves were collected from Rudsar city in Guilan province. After extraction by succulent method, concentrations of 0.5, 0.7, 1, 1.5, 2, 2.5, 5, 7.5, 25, 50 mg/ ml were prepared. The antioxidant capacity of the extracts was measured by DPPH method. The data were analyzed using one-way ANOVA. The results showed that aqueous and ethanolic extracts had the highest and ketone and ethanolic extracts had the lowest antioxidant capacity. The antioxidant capacity significantly decreased with increasing of extract concentration. The present study showed that the aqueous extract of mistletoe had higher antioxidant capacity than ethanol, acetone, and ether extraction. Increasing the concentration of the extract not only did not increase but also reduced the antioxidant capacity. Therefore, the use of aqueous extract with proper concentration is considerably important for the therapeutic purposes.

Keywords: Mistletoe plant, Extract, Antioxidant capacity

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1 Introduction

The mistletoe plant (*Viscum album*) is known as a medicinal plant. The extract of this plant is used to treat high blood pressure, epilepsy, diabetes, fatigue, anxiety and confusion, arthritis, dizziness and HIV infection (Pechter et al., 2018, Suveren et al., 2017; Szurpnicka et al., 2020). Antimicrobial, antiviral, an anticancer property of this plant have been also reported (Oei et al., 2019). Mistletoe is a common species in the Viscaceae family. It grows on other trees and contains a variety of biologically active substances. Its chemical composition may vary depending on harvest time, host tree species and production process. Alkaloids, flavonoids, tannins, anthraquinones, saponins and glucosides, amines, tripenoids, acetylcholine, lectin, and viscotoxins are the known phytochemical compounds found in mistletoe extract (Urech and Baumgartner, 2015). Among the mistletoe known chemicals, lectins and viscotoxins have been shown to play an essential role in the treatment of cancer. Phenolic acids, phenylpropanoids and flavonoids in mistletoe extract have antioxidant and anti-inflammatory activity. Mistletoe extract is also being considered as a liver protector and sedative (Nazaruk and Orlikowski, 2016).

Antioxidants play a pivotal role in combating against oxidative stress and preventing the diseases associated with oxidative stress. Free radical scavenging property of many plant extracts has been investigated in recent years. Mistletoe has been reported to have a high level of free radical scavenging property and antioxidant capacity (Tahirovic and Basik, 2017; Pietrzak et al., 2017) comparable to ascorbic acid (vitamin C). Ascorbic acid is a powerful antioxidant agent (Bilska et al., 2019).

In addition, no significant research has been done on the antioxidant capacity of mistletoe leaf extract grown in the forests of northern Iran, so the results of this study will be new in this regard.

Recent studies show that the diseases associated with oxidative stress are being increased worldwide (Banegas et al., 2018; Bertucci et al., 2019; Farhood et al., 2018; Mohammadi and Mirzaei, 2017) and impose an intolerable burden on patients and societies (McKinney and Smith, 2018). Therefore, investigation on plant extracts to find their antioxidant activity is an essential area of research. Contradictory findings have been reported on antioxidant capacity of mistletoe extract (Thronicke et al., 2020; Tsekouras and Kintzios, 2020) and few studies have been carried out to investigate the antioxidant capacity of mistletoe plant grown in the forests of northern Iran. The present study aimed to evaluate the antioxidant capacity of ethanol, acetone, ether and aqueous extract of mistletoe plant - growing in northern Iran - compared to vitamin C antioxidant capacity.

2 Materials and Methods

The mistletoe plant (*Viscum album*) leaves were collected from Gilan province, Rudsar, during 2017 and was identified by the botanists at IAU, Karaj Branch, Iran. The fresh leaves were sorted out to remove extraneous material and rinsed with water to remove debris and

dust particles. They were air dried and pulverized. For obtaining the aqueous extract, 50g of the powdered leaves was weighed into a beaker and 500ml of warm distilled water was added and stirred for 20 minutes. After 24 hours, the solution was filtered using Whatman filter paper to obtain the aqueous extract. To prepare the ethanol, ketone and ether extract, the powdered plant material (50 g) was subjected to extraction with ethanol , ketone (acetone) and ether, res[actively, at room temperature with occasional stirring. The samples were filtered and the ethanol, ketone and ether were removed at 40°C. The dried extracts were re-dissolved in water and 0.5, 0.7, 1, 1.5, 2, 2.5, 5, 7.5, 25, 50 microgram / ml of the extracts were prepared. Vitamin C was purchased form Karavaran Teb Asia Company, Iran. To obtain diffent concentrations of vitamin C, 1 g of vitamin C was mixed with 10 ml of distilled water and 0.5, 0.7, 1, 1.5, 2, 2.5. 5, 7.5, 25, and 50 microgram/ ml of vitamin C were prepared.

To measure antioxidant capacity of plant extracts the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was used. The DPPH radical-sc avenging activity was determined using the method proposed by Brand-Williams et al., (1995). The reaction was performed in 12 well-plate. A volume of 200 µl sample and 1.4 ml DPPH solution) were added to each microplate well. The percentage of scavenging effect of different extracts against DPPH radicals, was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_s) \times 100] / A_0 \quad (1)$$

Where, A_0 is absorbance of the blank, and A_s is absorbance of the samples at 515 nm.

SPSS software (version 20) was used for statistical analysis of data. Data analysis was performed by one-way ANOVA followed by Bonferroni post-test. Significance of differences between groups was considered at the level of $p < 0.05$.

3 Results and Discussions

The antioxidant capacity of 1 and 2 µg/ml of ethanol, ketone, ether, and aqueous mistletoe leave extract significantly increased compared to vitamin C antioxidant capacity ($p<0.001$) and the aqueous extract had the highest antioxidant capacity (Figure 1 and 2).

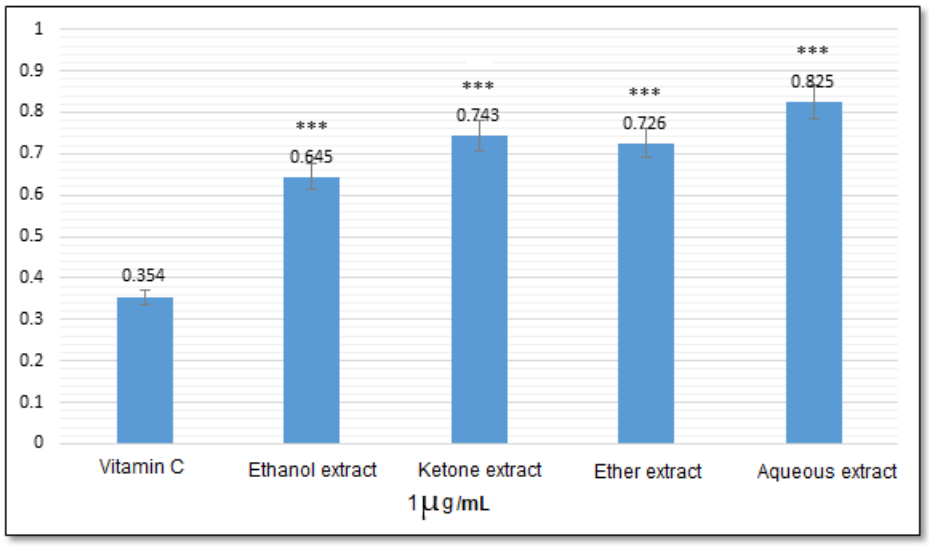

Figure 1. The antioxidant capacity of 1µg/ml of ethanol, ketone, ether, and aqueous mistletoe leave extract compared to vitamin C antioxidant capacity. *** ($p<0.001$) indicates significant difference compared to vitamin C, ethanol, and ether extract, respectively.

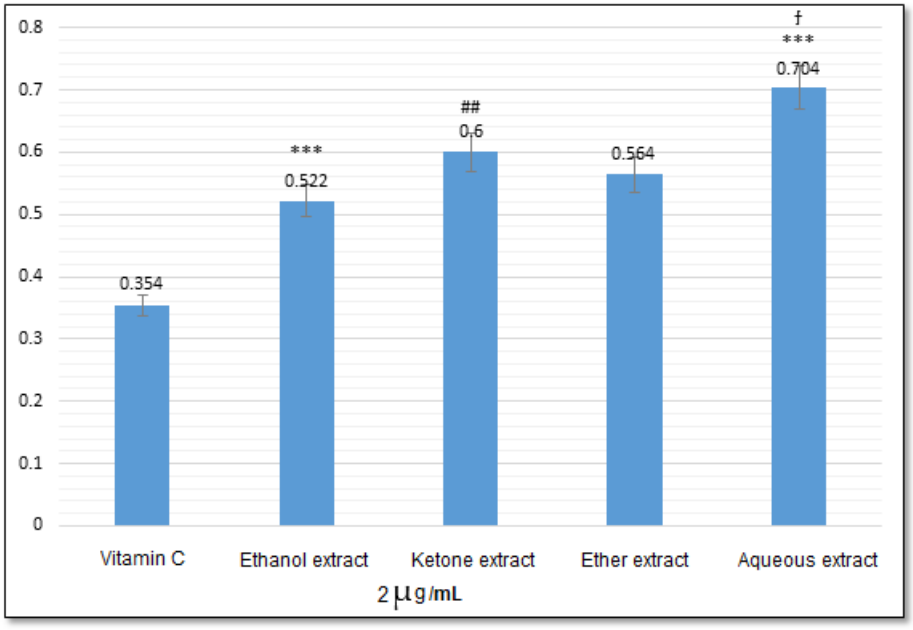

Figure 2. The antioxidant capacity of 2µg/ml of ethanol, ketone, ether, and aqueous mistletoe leave extract compared to vitamin C antioxidant capacity. *** ($p<0.001$) indicates significant difference compared to vitamin C, ethanol, and ether extract, respectively.

The antioxidant capacity of 5, 7.5 and 25 µg/ml of ethanol, ketone, and ether extracts significantly reduced ($p<0.001$) and of aqueous extract significantly increased ($p<0.001$) compared to vitamin C antioxidant capacity (Figure 3, 4 and 5).

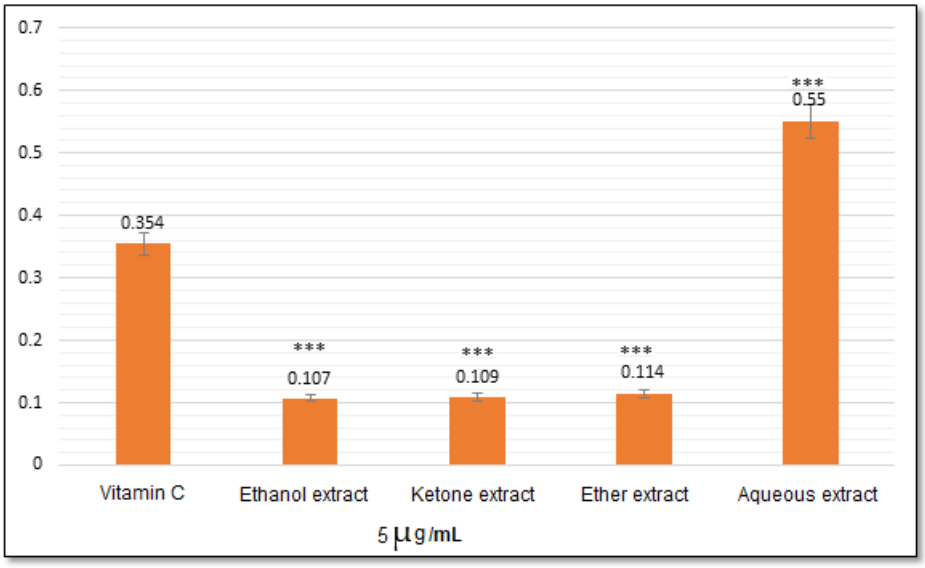

Figure 3. The antioxidant capacity of 5µg/ml of ethanol, ketone, ether, and aqueous mistletoe leave extract compared to vitamin C antioxidant capacity. *** ($p<0.001$) indicates significant difference compared to vitamin C, ethanol, and ether extract, respectively.

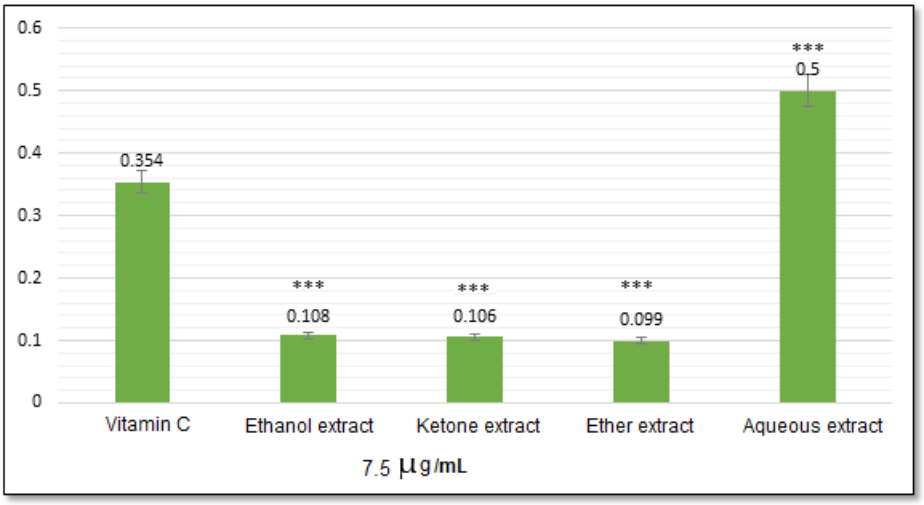

Figure 4. The antioxidant capacity of 7.5µg/ml of ethanol, ketone, ether, and aqueous mistletoe leave extract compared to vitamin C antioxidant capacity. *** ($p<0.001$) indicates significant difference compared to vitamin C, ethanol, and ether extract, respectively.

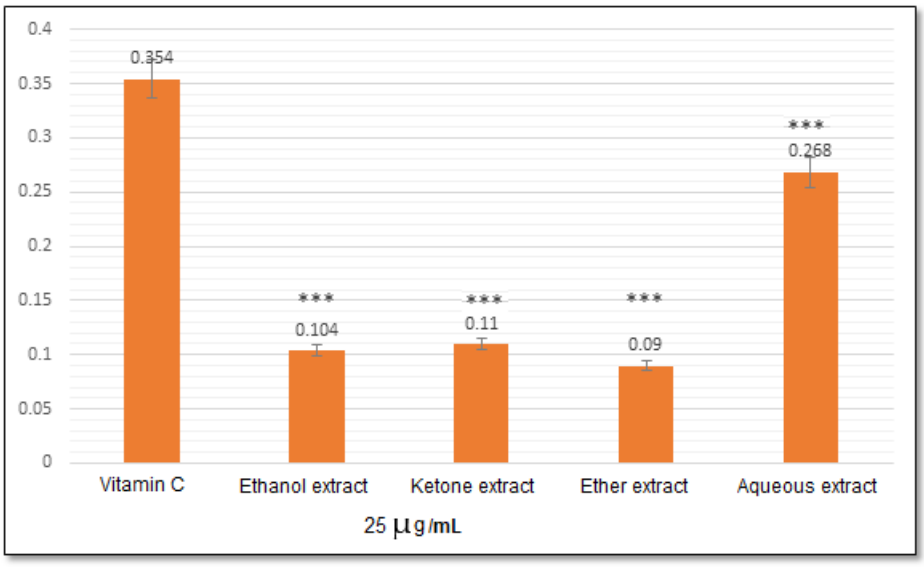

Figure 5. The antioxidant capacity of 25 µg/ml of ethanol, ketone, ether, and aqueous mistletoe leave extract compared to vitamin C antioxidant capacity. *** ($p<0.001$) indicates significant difference compared to vitamin C, ethanol, and ether extract, respectively.

The antioxidant capacity of 50 µg/ml of ethanol, ketone, ether and aqueous extracts significantly reduced ($p<0.001$) compared to vitamin C antioxidant capacity (Figure 6).

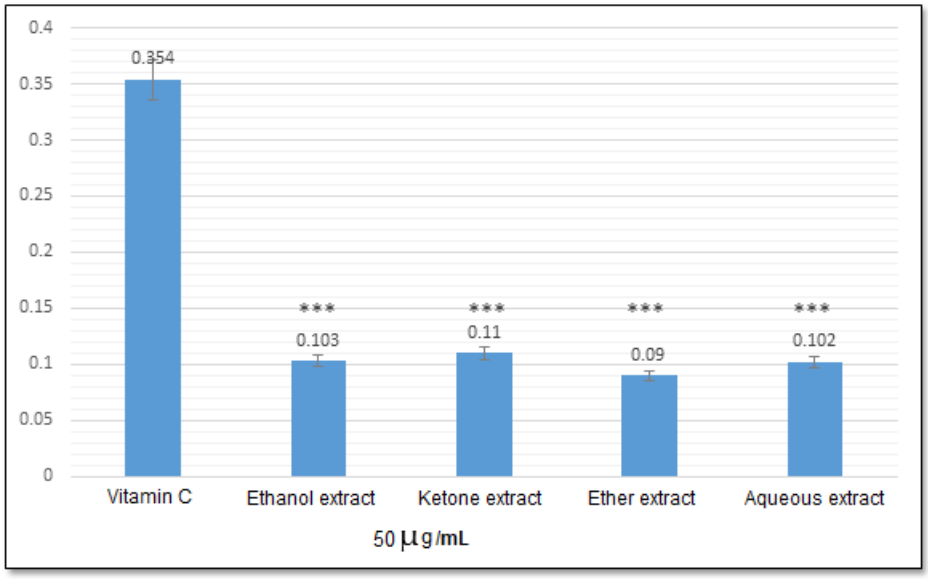

Figure 6. The antioxidant capacity of 50 µg/ml of ethanol, ketone, ether, and aqueous mistletoe leave extract compared to vitamin C antioxidant capacity. *** ($p<0.001$) indicates significant difference compared to vitamin C, ethanol, and ether extract, respectively.

The results of this study show that the antioxidant capacity of lower concentration (1 and 2 µg/ml) of ethanol, ketone, ether and aqueous extracts of mistletoe leaves were higher than the antioxidant capacity of vitamin C, among them, the aqueous extract had the highest antioxidant capacity; however, the increased concentration (5, 7.5 and 25 µg/ml) of the extract resulted in

significant decrease in antioxidant capacity of ethanol, ketone, and ether (but not aqueous) extracts compared with vitamin C antioxidant capacity. Consistent with these findings, mistletoe has been reported to have a high level of free radical scavenging property and antioxidant capacity (Tahirovic and Basik, 2017; Pietrzak et al., 2017). In a study scavenging activity of methanolic extracts of *Viscum album* was determined by DPPH method and the extract from mistletoe grown on lime tree in summer has been reported to have the highest antioxidant activity. It was found that antioxidant capacity of the plant differed according to the harvesting time as well as the host tree (ÖnayUçar et al., 2006). The mistletoe alcoholic extract is also able to annihilate oxygen free radicals (superoxide anion, hydroxyl radical, hydrogen peroxide and nitric oxide) (Papuc et al., 2010). The chemometric analysis indicated that mistletoe had the most advantageous chemical composition and antioxidant activity (Pietrzak et al., 2021). It has been shown that crude extracts and chemical compounds isolated from mistletoe have significant antioxidant and medicinal effects in experimental models and in patients with cancer, hypertension and show antioxidant, cytotoxic, anti-tumor, anti-inflammatory, anti-diabetic, antimicrobial, and sedative activities. Lectins, viscotoxins, heterodimer glycoproteins, polysaccharides, alkaloids, lipids, triterpenes, peptides, vesicles, flavonoids, cyclitols and amines are chemically active substances found in mistletoe extract (Singh et al., 2016). Also, mistletoe extract bioactive substances with cytotoxic properties can be useful for treatment of cancer (Mavrikou et al., 2020; Kim et al., 2020; Felenda et al., 2019; Kenar et al., 2016; Twardziok et al., 2016), at least in part, because of their antioxidant capacity. By contrast, findings of a study revealed that mistletoe extract is not useful in cancer treatment (Freuding et al., 2019).

We have also shown that the higher concentration (50 μg/ml) of the ethanol, ketone, ether, and even aqueous mistletoe leaves extracts had significantly lower antioxidant capacity than vitamin C, indicating that the higher concentrations of mistletoe leaves extracts may have no significant beneficial effects on treatment of diseases or even may have cytotoxic effects on normal cells due to their lower antioxidant capacity.

4 Conclusion

The results of this study indicate that lower concentrations of aqueous extract of mistletoe leaves collected from northern Iran show a higher level of free radical scavenging property and antioxidant capacity than vitamin C antioxidant capacity; according to which, the aqueous mistletoe leaves extract may to be beneficial in the treatment of metabolic diseases.

Acknowledgments

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Conflict of interests

The authors state that there are no conflicts of interest regarding the publication of this article.

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