

## The Anti-Radical Properties of Phenolic Compounds of Dry and Fresh Barberry (*Berberis Integerrima* × *Vulgaris*)

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### ABSTRACT

Barberry belongs to the *Berberidaceae* that its used in traditional medicine has a long history. The aim of this study was to compare the capacity of nitrite radical gathering, superoxide, Malondialdehyde and power reduction in ethanol, methanol and water extracts of hybrid *Berberis Integerrima* × *Vulgaris*. The aqueous extract of fresh barberries the maximum capacity of radical nitrite gathering ( $723/60 \pm 64/56$ ) and the maximum capacity of superoxide radical gathering ( $202/98 \pm 1/75$ ). The ethanol extract of dried barberries the maximum power reduction ( $0/37 \pm 0/006$ ) and The methanol extract of fresh barberries maximum amount of MDA ( $37/12 \pm 0/79$  mmol/g), was obtained. The results showed that barberry fruits have high Anti-oxidative capacity which can be used as a plant source in the food and pharmaceutical Industries.

**Keywords:** Anti-Radical properties, phenolic compounds, Barberry

### INTRODUCTION

Antioxidants by neutralization of free radicals contribute to not only the decrease of cardiovascular disease and heart attacks but also to the prevention of cancer growth. In addition, antioxidants protect the cell membrane (Lien et al, 2008). Immune system isn't able to eradicate free radicals by the existence of different antioxidants in the body and for this process needs to get help of the antioxidants in the food (Halliwall and Gutterige, 1998). It's proved that antioxidants which exist in some nutrients such as butylated Hydroxyanisole, Tert-butyl hydroxyl toluene and beta hydroxyl-Quinone are poisonous and cause malnutrition. So, nowadays, nutritionists advise the consumption of fruit and vegetables in order to provide the necessary antioxidants for the body. The consumption of vegetal antioxidants has lesser side effects and is more

beneficial (Franklin, 1991). There is a wide range of secondary metabolites in vegetables which protects us against environmental challenges either alive or dead (Dixon, 2001). Fruit and vegetables have been studied in different countries in order to investigate the existence of phenol and anti-oxidation activity. It is proved that the consumption of fruit and vegetables contributes to the eradication of cardiovascular disease, different cancers and senescence (Mohammad et al 2007; Yang et al, 2009; Yu et al, 2005). When the inhibitory mechanism of body which consists of anti-oxidation system fails, free radicals cause cancer, atherosclerosis and other physiologic disorders (Abe et al 2010). Therefore, it is essential to receive anti-oxidative compounds through eating. Among derived antioxidants from vegetables, phenols and flavonoids have strong activity in collecting ROS and decrease the risks of cardiovascular disease. There are phenolic compounds in both food and

non-food parts of vegetables and they have a variety of biological effects such as the capacity of free radical gathering (Rozan et al, 2007).

One of the most important destructive effects of free radicals is lipid peroxidation which cause the destruction of cell membrane the oxidation of unsaturated fatty acids leads to the reduction of membrane fluidity and the destruction of its structure and function. So the small amounts of antioxidants in immune system protects cell membrane and different compounds of a creature against oxidants (Rice –Evans and Burdon 1994). Barberry is full of different minerals and vitamins and its nutritional value is high. Barberry's vitamin C is more than lemon's (Kafi and Balandri, 1381). Barberry consists of different minerals such as: Tron, calcium, magnesium, sodium, potassium, copper, zinc, manganese, phosphor, lipid, protein, reducing sugars and anthocyanins. (Berenji Ardestani et al, 2010). Biological compounds of barberry are used in food and medical industries the usage of barberry as a natural colorants instead of artificial and harmful colorants has been studied (Sharifi et al 2007). Barberry has been used in different ways for a long time in traditional medicine of Iran (Zargari, 1993; Fatehi –Hassanabad et al, 2008). Berberine is one of this plant's compounds which can prevent coronary artery disorders and eventually decrease the level of total cholesterol and triglyceride (Kong et al, 2004). Important chemical components of barberry are Berberine, Oxyacanthine and Berbamine. Berberine is the main alkaloid which is separated from barberry's root and skin. Barberry's extract consists of different flavonoids like quercetin, Chrysanthemin, Hayprvzyd, delphinidin-3-o-Beta-D-glucoside, Plargvny and Petunidin-3-O-Beta-D-Glucoside (Gilgon- Sherki et al, 2001). Besides, it consists of anti-oxidative compounds like ascorbic acid, alpha –Tocopherol and beta-carotene (Ferre et al, 2001). Nowadays, because of bad effects of synthetic antioxidants, there is an increasing tendency to the consuming of natural antioxidants. therefore, in this study, the capacity of anti-radicalism of dry and fresh barberry in water, methanol and ethanol extracts has been compared in order to find the best conditions of keeping barberry. Barberry could be used in different areas

such as food, medical and pharmaceutical industries.

## METHODS AND MATERIALS

### Plant gathering

*Berberis integerrima\* vulgaris* was gathered in November 2019 from Qamchoqai zone in Bijar and it's preserved in freezer till the evaluation time.

### Extract preparation

The different extracts of barberry were prepared by maceration. 1g of dry and 2g for frozen barberry was grinded and it was completely squashed and finally it was mixed with 25ml methanol for 2 hours in a shaker. This mixture was passed filters and this extract was kept in special tube away from sunlight in the refrigerator. For preparing water and ethanol extracts, above procedures were repeated.

### The capacity of nitrite radical gathering

The amount of Nitrite radicals was determined by Griess-Ilosvoy reaction (Garrat, 1964). 2 ml Nitroprusside Sodium (10 mmol) and 0/5 ml Phosphate Buffered Saline was mixed with 20 µl of extract and it was incubated for 150 minutes in the room temperature. After incubation, 1 ml of it was mixed with Sulfanilic Acid (0/33 in glacial Acetic Acid %20) and it was left for 5 minutes. Then, 1ml Naphthyl Ethylene Diamine Hydrochloride 0/1 % was added to it and after 30 minutes the amount of absorption was measured by a Spectrophotometer (at 540 nm). The percentage of radical gathering was calculated by below equation:

$$\text{Percent of Nitrite radical gathering} = \frac{(A_{\text{blank}} - A_{\text{sample}}) * 100}{A_{\text{sample}}}$$

### Determining of reduction power

The power of extracts for Iron reduction was measured by Yildirim et al (2001) method with a little change. For measuring the reduction power, 2/5 ml Ferro cyanide and 2/5 ml TCA was added to 100µl extract 2/5 ml Buffer (ph.=6/6). Then, it was put in a Ben Murray for 30 minutes. 0/5 ml FeCl<sub>3</sub> was solved in 2 ml distilled water in attest tube. Then, 2 ml of previous solvent was added to this

solvent and the amount of absorption was measured at 700 nm. The high amount of absorption, the high amount of reduction power(Oyaizu, 1986).

**Determining of Lipid Peroxidation by Thiobarbitoric Acid method**

TBA method was used for the measurement of Lipid Peroxidation. It's bonded to TBA at 100C and low PH and a red beverage was formed which can be determined at 432 nm. 2ml TCA 20% and 2ml TBA 67% was added to 0/5 ml of extracts. This solution was put in Ben Murray for 20

minutes at 100 C. after that it was reached to room temperature, it was centrifuged for 20 minutes with 3000Rpm. Anti-oxidative activity based on Zincoid solution, absorption is at 532 nm. The amount was determined by below equation:

$$MDA/GFW = (\text{absorption} / 0/55) * 1000 * \text{dilution times.}$$

**The capacity of Superoxide radical gathering**

The amount of superoxide radical gathering was determined by Pyrvgall system and Jing et al (1995) method. 9ml Tris Buffer-

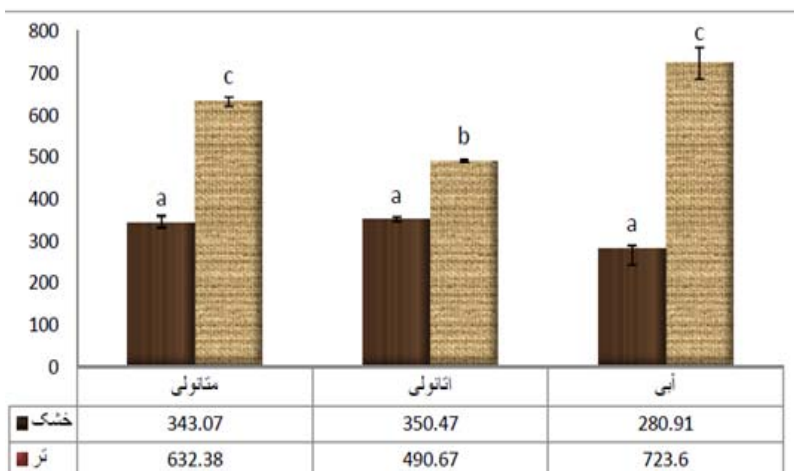


Fig. 1: the percentage of Nitrite radical gathering in fresh and dry Barberry in different extracts(methanol, ethanol and water), (p<0/05,SE= 3times)

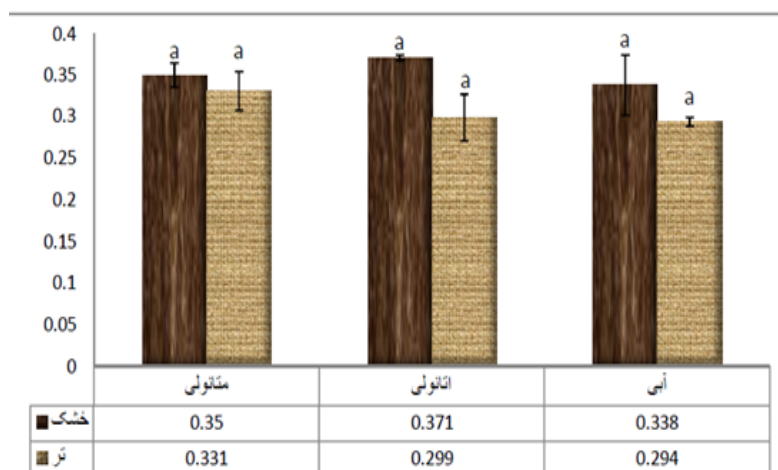


Fig. 2: reduction power at 700 nm in fresh and dry barberry extracts (ethanol, methanol and water), (p<0/05 and SE= 3 times)

Hydrochloric Acid (ph=8/2 and 50 mmol/l) was added to the tube test. This tube was incubated in a warm bath at 25 C for 20 minutes. 40  $\mu$ l of Pyrv gall solution (45mmol\* HCL 10mmol/l) which had been incubated at 25 C before adding to the tube test. This solution was incubated at 25 C for 3 minutes and then one drop of Ascrobic Acid was added to that solution. The absorption at 420 nm after 5 minutes as  $A_0$  was measured. The speed of auto oxidation ( $A_1$ ) was measured by Pyrv gall method. This time, certain amount of extract was added to Tris Buffer-HCl solution. Finally, a control factor as  $A_2$  was determined. The percentage of superoxide radical gathering was determined by below formula

$$\text{The percentage of superoxide radical gathering} = A_0 - (A_1 - A_2) * 100 / A_0$$

#### Statistical analysis

All tests were repeated for three times and results were shown in form of average and standard deviation (SD). The variation between samples was defined and analyzed by bilinear variance ( $p < 5\%$ ). Tables and diagrams were drawn by SPSS and EXCEL software.

#### Discussion and results

##### The capacity of Nitrite radical gathering

According to the results which are shown in diagram 1, fresh barberry in comparison with dry

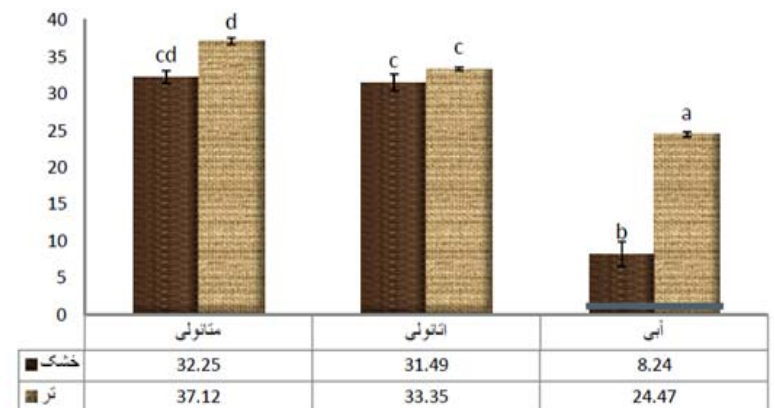


Fig. 3: the amount of MDA in methanol, ethanol and water extracts of fresh and dry barberry ( $p < 0/05$ , SE= 3 times)

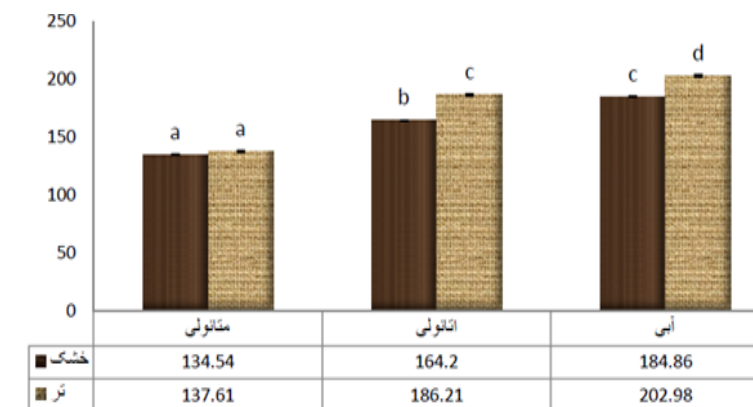


Fig. 4: the percentage of superoxide radical gathering in the methanol, ethanol and water extracts of fresh and dry barberry ( $p < 0/05$ , SE +/- 3 times)

barberry has more capacity of Nitrite oxide gathering. The maximum amount of Nitrite radical was in water extract of fresh barberry (723/60+64/56+) and the minimum amount of Nitrite radical gathering was in water extract of dry barberry (280/91=-15/41). Nitrite radical gathering activity depends on the density of the extract (Komaran and Karunakaran, 2008). NO and NO<sub>2</sub> because of having unpaired electron are free radicals. NO is a strong inhibitor in some physiologic procedures such as: controlling blood pressure, relaxing muscles, transferring neural signals, aggregation of platelets and having antibacterial and anticancer properties. (Hageman et al, 1998). It is proved that plants use NO as a growth regulator which moderates anti-bacterial reactions (Sakamoto et al, 2002). Recently, it's proved that this substance plays a vital and important role in the regulation of normal physiologic activities of plants like stomatal closure, growth and evolvment (Zhua et al, 2006).

In Jiao et al (2008) and Nikkhah et al (2009) studies, Phenolic compounds are good sources of antioxidants. The more extract's dose is, the more scavenging percent of phenolic compounds. It's shown in Sasikumar et al (2012) studies that different densities of barberry's extracts have a good inhibitory potential in comparison with Nitric oxide radical. In this study, the results are the same.

#### **Determining of reduction power**

The reduction power of methanol, ethanol and water extracts of fresh and dry barberry has been measured by transformation of Fe<sup>3+</sup> to Fe<sup>2+</sup> (diagram 2). There wasn't any difference in reduction power of different extracts (absorption at 700nm). The maximum amount of reduction power was in the ethanol extract of dry barberry (0/371+-0/0061) and the minimum amount of reduction power was in water extract of fresh barberry (0/294+-0/009). The iron reduction method is the fastest and the best way for measuring the reduction power of chemical compounds and it can be used as an index of antioxidant power of chemical compounds (Gulcin, 2009). Antioxidants with a high reduction power of iron have a good ability in ending the destructive chain reactions of free radicals' formation (Wanasundara and Shahidi, 2005). In this method, reduction power of nutrients in the sample is measured by reducing Fe<sup>3+</sup> to Fe<sup>2+</sup> (Chung et al,

2006). The reduction of iron 3 is mainly used as the index of electron activity (Hinneburg et al, 2006). Different extracts of barberry have a high reduction power (Mohammadi et al, 2012). The reduction power of methanol extract is less than BHT (Sasikumar et al, 2012). The potential of reduction power of fresh barberry is more than the dry barberry (Beyhan et al, 2010). The results showed that the reduction power of barberry extract is because of the polyphenol's presence that these polyphenols act as a reduction.

#### **Determining of Lipid Peroxidation by Thiobarbitoric Acid method**

The measurement of MDA in methanol, ethanol and water extracts of dry and fresh barberry is shown in diagram 3. The maximum amount of MDA was in methanol extract of fresh barberry (378/12+-0/79) and the minimum amount of MDA was in water extract of dry barberry (8/24+-0/6). In both dry and fresh barberries, methanol and water extracts have the maximum and minimum amounts of MDA. The amount of Lipid Peroxidation in tissues was measured by Thiobarbitoric Acid method. When oxidative tension occurs, Lipid Peroxidation of unsaturated fatty acids increases. Because of the free radicals' attack to Lipids, different Aldehydes such as MDA will be created (Mates et al, 1999). The production of Lipid Peroxidation with a high and free radical is a sign of a toxic condition and its final product is MDA. The increased MDA is an index for physiologic tension (Pourakbar et al, 2007). This anti-oxidative effect is because of the phenolic extracts. As a matter of fact, by increasing the density, the amount of phenolic compounds increases and this causes the inhibitory of free radicals.

These compounds because of having Hydroxyl group have the ability of free radicals' neutralization. By adding the skin and the seed of grape's extract to the sunflower oil, Emad (2006) understood that the anti-oxidative power of the grape's skin is more than its seed. In other words, phenolic compounds by cleansing free radicals prevent from Lipid Oxidation and the MDA increases. In total, barberry's extract as a source of natural antioxidants has the ability to react against free radicals. In this study, the amount of MDA had a reverse ratio with the amount of total phenol in different extracts of the barberry. i.e. methanol

extracts had the maximum and water extracts had the minimum amount of MDA.

#### The capacity of superoxide radical gathering

For measuring the capacity of superoxide radical gathering, different extracts of fresh and dry barberry were studied by Pyrvigall method. According to the given data in diagram 4, the maximum amount of SO was in water extract of fresh barberry (202/98-+1/75) and the minimum amount of SO was in methanol extract of dry barberry (134/54+-0/59). SO radical is one of the most dangerous free radicals in human body (Schlesier et al, 2002). So radical is so toxic that it is a sub-product of Mitochondrial respiration. The capacity of SO radical gathering was studied by Pyrvigall oxidation system. The capacity of SO radical gathering in different densities of methanol extract of barberry has been studied in comparison with BHT. The amount of radical gathering in barberry's extract was significantly more than the BHT's synthetic antioxidant. By increasing the sample's density, the reducing power of SO radical increases (Jiao et al, 2005). In this study, the anti-radical activity

of different extracts (specially the fresh barberry's extract) was good.

#### CONCLUSION

In different studies, it is proved that phenolic compounds like flavonoids have the anti-oxidative and anti-radical effects such as: the capacity of Nitrite oxide radical gathering, superoxide and anti-oxidative activities. It is concluded that the fresh barberry is rich of phenolic compounds and it can be used in both food and pharmaceutical industries. The amount of nitrite radical gathering, MDA and SO radical gathering in fresh barberry was more than in the dry barberry, but there was no significant difference in reducing power. In other words, we recommend the use of both fresh and dry barberry. Anyway, both fresh and dry barberry have the anti-oxidative activity and are introduced as a source of phenolic compounds. Based on the results, different extracts have a high nutritional value, but the use of water extract is highly recommended because it is harmless.

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