Radical Scavenging Potential of *Iranian Quercus Brantii and Juglans Regia*

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Abstract: Antioxidant activity of hydro- alcoholic leave extracts of Quercus *brantii* and *Juglan sregia* were evaluated. For antioxidant determination used Superoxide anion, hydroxyl radical,nitric oxide radical scavenging activity and metal chelating potential in different concentration(.25, 0.5, 1,2 and 4mg/ml) by in vitro method**. Material and Methods:** Hydroxyl scavenging activity in *Juglans regia* (19-63%) was less than *Quercus brantii* extracts (23-74). IC50 value of *Quercus brantii* and *Juglans regia* were reported1.57±0.05 and 2.43±0.5mg/ml respectively. **Results:** Superoxide radical Inhibition in *Quercus brantii* and *Juglans regia* were observed 25-64 and 21-54 respectively. IC_{50} of *Quercus brantii* (2.1±0.02) and *Juglans regia* (3.26± 0.07) mg/ml were reported respectively. The maximum chelating activity (% of inhibition) of *Juglans regia* (56) and *Quercus brantii* (53) were reported in the highest concentration .The IC_{50} values of *Quercus brantii* and *Juglans regia* for Chelating activity reported 3.5±0.09 and 03.17± 0.02 respectively. Inhibition in nitric oxide in *Juglans regia* (34- 66) and *Quercus brantii*(27-56) were observed respectively. The IC50 values of *Quercus brantii* and *Juglans regia* for nitric oxide scavenging were reported 2.35 ±0.04and 1.32 ±0.08respectively. **Conclusion:** Hydroxyl and Superoxide radical scavenging activity of *Quercus brantii* was reported more than *Juglans regia* extract. *Quercus brantii* and especially *Juglans regia* extracts could be used in the treatment of iron-overload disorders due to its high chelating potential at low doses

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Key words: *Quercus brantii, Juglans regia*, antioxidant activity, scavenging activity

1. Introduction

Free radicals are generate biochemical damages to cells, tissues and result informed many age related degenerative diseases including atherosclerosis, arthritis, heart disease, retinal damage, stroke and cancer(RajeshMandade, 2011). They can produce lipid peroxidation and attacks to proteins and DNA and induce damages too (Blomhoff, 2006;Packer,2001). Antioxidants are compounds with neutralize of free radical potential and reduce oxidation damage thus, protecting organisms from damages (Packer, 2001). According to the World Health Organization, approximately 80 per cent of world people still use mainly on traditional remedies for their medicines and treatment of disease (Canadanovic-Brunet, 2001). Medicinal plants are frequently used for prevention and cure of ailment .They may besourcesfor new drug and healthcare products which called Phytochemicals (Rahman, 2008). The antioxidant capacity of phenolic compound is mostlydue to their redox potential. These activities contain reducing potential, metal chelating, hydrogen donors and free radical scavenging (Marimuthu, 2008). Antioxidant activities due to phenolic compounds also show a wide range of medicinal properties, such asanti-allergic, anti-inflammatory, anti-microbial, anti-thrombotic and cardio-protective effects (Balasundram, 2006). There are about 2,500000 species of medicinal plant and most of them not been studied for their biological activity such as antioxidant potentials (Ram, 2004).

Quercus species is distributed dincentral and southeast part of Iran. It used for treatments of intestinal disorders, inflammation and diarrhea (Rocha-Guzman, 2007). Antioxidant and anti-lipid peroxidation of leaves and fruit components *Quercus* species was reported in literature (Seddik, 2010). *Juglans regia (Juglans regia L*) is a member of *Juglandaceae* family which used for cure of hypercholesterolemia andhypertensive disease. Some biological activity such as antibacterialand antifungal properties of *Juglans regia* was reported by researchers. Linoleic, linoleic and oleic acid, high protein, carbohydrate and vitamins were seen in *Juglans regia*nut (Ogunmoyole, 2011). The objective of the present research was to investigate leaves of *Juglan sregia* and *Quercus brantii plants* antioxidant activities. For determination of antioxidant potential four free radical scavenging activity such as metal chelating potential, superoxide anion, and nitric oxide and hydroxyl radical scavenging activity were used.

2.1Material and Methods

2.1.*Plant collection and extraction*

Leaves of the *Quercus brantii* and *Juglans regia* were collected in Yasuj. Samples were identified and avoucher specimen was placed in thebiochemistry laboratory, Yasuj University of Medical Sciences, Yasuj, Iran. After washed the samples and shade-dried, the hydro- alcoholic extraction was accomplished by maceration in 48 h. at room temperature.

2.2.*Metal chelating activity*

The chelation of Fe^{2+} ions by extracts was determined using modified method of Dinis (Dinis, 1994.

2.3.*Superoxide anion scavenging activity assay*

The scavenging activity of the extracts against superoxide anion radicals was determined based on the method described by Winterbourne (Yu, 2004).

2.4.*Nitric oxide radical inhibition assay*

Nitric oxide radical scavenging activity of plant extract was measured by the use of Griess Illosvoy reaction (Olabinri, 2010).

2.5.*Hydroxyl radical scavenging activity*

The hydroxyl radical scavenging activity was measured by method which described Yu (Winterbourne, 1975).

2.6. *Statistical analysis*

Tests were carried out in triplicate for experiments. The quantity of extract required to inhibit free radicals level by 50%, IC50, was graphically calculated using a linear regression algorithm.

3. Results

In present investigating there was recorded a dose dependent response in metal chelating, Super oxide, nitric oxide and hydroxyl radical scavenging activity with different concentrations. The maximum chelating activity (% of inhibition) of *Juglansregia* extracts (56%) was established as higher than that of *Quercus brantii* extracts (53%) in concentration 4 mg/ml .The IC_{50} values of *Quercus brantii* and *Juglans regia* for Chelating activity reported 3.5±0.09 and 03.17± 0.02 respectively. The lowest chelating activities were obtained from *Quercus brantii* and *Juglans regia* 18 % and 21 respectively (Figure 1) (Table 1). The superoxide free radical scavenging activity of *Quercus brantii* and *Juglans regia* extract assayed was shown in(Figure 2) (Table 1). The superoxide scavenging activity of *Quercus brantii* and *Juglans regia* was increased markedly with the increase in concentrations. The Inhibition percentage of Superoxide free radicals in range concentration (.25-4 mg/ml) from *Quercus brantii* and *Juglans regia* were observed 25-64 and 21-54 respectively. The half inhibition concentration (IC_{50}) of *Quercus brantii* (2.1±0.02) and *Juglans regia* (3.26± 0.07) mg/ml were reported.The percentage of inhibition in nitric oxide by different concentration (.25, 0.5, 1,2 and 4 mg/ml) in *Juglan sregia* were observed 34, 42, 59, 63 and 66 respectively .whereas the percentage inhibition of *Quercus brantii* in same concentration was found to be 27, 36, 46, 51 and 56respectively. The IC_{50} values of *Quercus brantii* and *Juglans regia* for nitric oxide scavenging were reported 2.35 ±0.04and 1.32 ±0.08respectively (Figure 3) (Table 1). Hydroxyl scavenging assay recorded a dose dependent effect of *Quercus brantii* (23-74 mg/ml) (Figure.), with an IC_{50} value of 1.57±0.05 mg/ml that was comparable with IC_{50} value of 2.43±0.5 m/ml observed in *Juglans regia* (Table1). Hydroxyl scavenging activity in *Juglans regia* was less than *Quercus brantii* extracts (19-63%) (Figure 1).

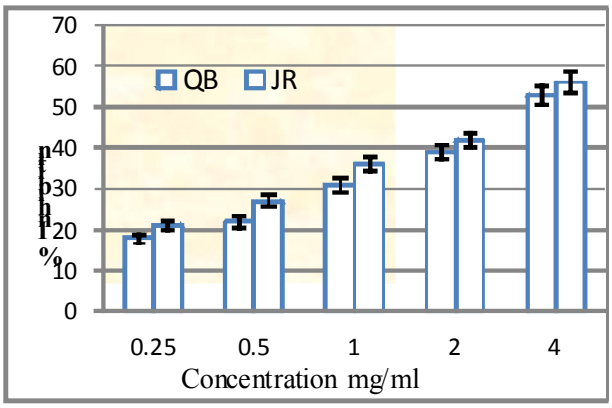

Fig. 1: Metal chelating activitieswith different concentrations in hydro – alcoholic extract of*Quercusbrantii (QB)*and *Juglansregia* (JR). Each value is expressed as mean ± standard deviation (n = 3).Significant (p <0.05).

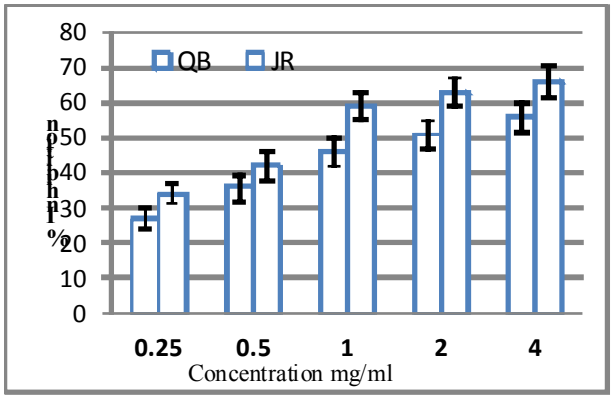

Fig. 2: Nitric oxide radical scavenging activitieswith different concentrations in hydro – alcoholic extract of*Quercusbrantii (QB)*and *Juglansregia* (JR). Each value is expressed as mean ± standard deviation (n = 3).Significant (p <0.05).

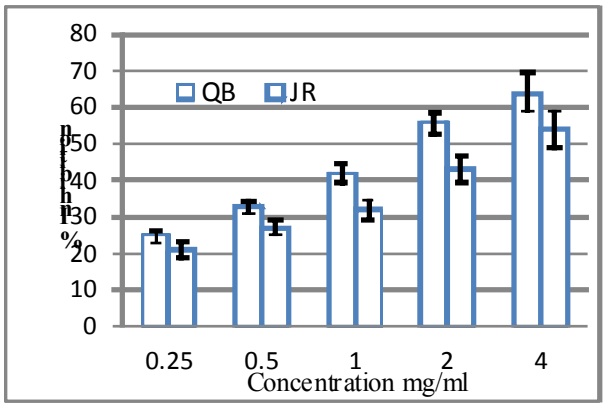

Fig. 3: Super oxide radical scavenging activities with different concentrations in hydro – alcoholic extract of *Quercusbrantii (QB)* and *Juglansregia* (JR). Each value is expressed as mean ± standard deviation (n = 3). Significant (p <0.05).

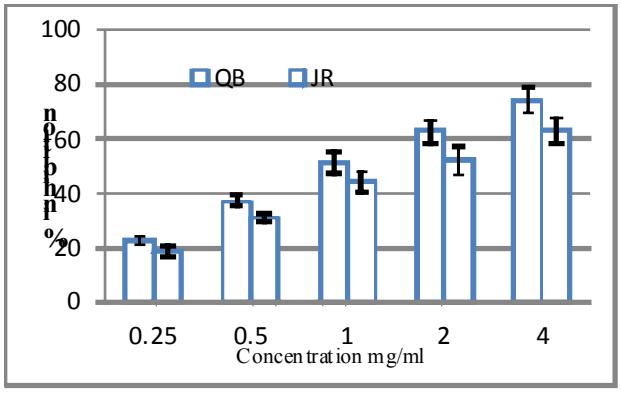

Fig. 4: Hydroxyl radical scavenging potentials withdifferent concentrations in hydro – alcoholic extract of*Quercusbrantii (QB)*and*Juglansregia* (JR). Each value is expressed as mean ± standard deviation (n = 3).Significant (p <0.05).

Table 1: Fifty percent of inhibitory concentration (IC_{50}) indifferent concentrations in hydro – alcoholic extracts of*Quercusbrantii (QB)* and*Juglansregia* (JR).

Assay	IC_{50} (mg/ml)	
	JR	*QB*
Superoxide radical scavenging	3.26 ±0.07	2.1 ±0.02
Hydroxyl radical scavenging	2.43 ±0.5	1.57 ±0.05
Nitric oxide radical scavenging	1.32 ±0.08	2.35 ±0.04
Metal chelating	3.17 ±0.02	3.5 ±0.09

Estimation of IC_{50} in Superoxide anion, hydroxyl radical, nitric oxide radical scavenging activity, and metal chelating potential with concentration of 0.25, 0.5, 1, 2and 4 mg/ml. Significant (p <0.05).

4. Discussion

Now, free radicals are related with numerous degenerative diseases such as cancer, heart diseases, rheumatoid arthritis and others. Antioxidants induced defend against free radical generation and injury induced by lipid peroxidation, protein damage and DNA breach. Phenolic compounds such as total phenols and flavonoids are the important group of antioxidant substances that related to quality and food value. They have naturalized free radicals such as peroxide, hydro peroxide and metal chelating (Kumaraswamy, 2008) (Navnath, 2010).

There is a reverse relationship between high intake dietary antioxidant and the incidence of human disorders (Satheeshkumar, 2010). It has been documented that high intake of phenolic compound revealed inhibitory potential on mutagenesis and carcinogen development. Chelation activity may useful for iron-overload treatment and induce Protection against oxidative stress (Lai, 2001). Chelating potential in recent study was dose-dependent and capable inhibited of free-radical generation in biological systems .Metal chelating activity in plant extracts may be due to presence of their total phenol compounds and antioxidant activities (Michalak, 2006). Hence, metal chelation was the major property of antioxidant activity in plants. Excretion of iron in different forms such as soluble and stable complexes was increased in urine and feces by iron chelators in chelation therapy. Therefore, plasma iron concentration and iron overload complications was reduced and increased quality of life in human body. In Thalassemia and Alzheimer's disease iron overload is a major problems. Consequently, iron chelation is a necessary therapeutic strategy for survival (Reznichenko, 2006). Oxidation and hydroxyl radical's formations are often producing by transition metal in foods via Fenton reaction. Iron chelation by Plants could be delayed these reactions. Transition metals such as iron and copper in optimum concentration are very important in some biological activity including cellular respiration, blood gas transport, enzyme activity and redox reactions. However, iron and cop-per are very reactive metals and possessone or more unpaired electrons in outermost electron layer therefore, they are most potent catalysts for oxidation reaction and free radical generation and lipid peroxidation via the Fenton and Haber-weiss reaction in live organism. Some plant extracts with antioxidant activity have exhibited metal chelating potential andinhibited of lipid peroxidation. For evaluation of this property determination of iron

chelating is essential step (Duh, 1999) (Kessler, 2003). According to present result the plant extracts have iron chelating potential which might be due to the presences of total phenol compounds .They have prevented the cell from free radical injury by decreasing of transition metal ions (Duh, 1999). Different plant extracts were demonstrated to be good chelators and association present between total phenols, flavonoids and chelating potential.

Super oxide Dismutase (SOD) is an antioxidant enzyme that present in live organisms. It guards the cell and tissues from damages which induced by Superoxideradicals. SOD reduces superoxide radicals that generated by cell injuries via conversion of superoxide radicals into hydrogen peroxide (Rajesh Mandade, 2011). Superoxide radical is a very injurious species for body cellular and produced hydrogen peroxide through dismutation reaction. Furthermore, the hydroxyl radical generation from hydrogen peroxide and lipid peroxidation are unfavorable effects of superoxide radicals in human body. Superoxide scavenging capacity of plant extract may be mostly due to the presence of secondary phytochemicals such as total phenol and flavonoids. The present extracts were shown an effective scavenger of superoxide radical that produced in PMS-NADH system in vitro and their potential are in similar to literature (Okoronkwo, 2010). This result obviously revealed that the tested extracts significant effect on superoxide anion formation were noted (Rajesh Mandade, 2011).

Nitric oxide (NO) is a very useful mediator in some physiological and biologic pathways in human body. Its antioxidant and antitumor potential were reported in literature. It reacts with superoxide anion and generated peroxynitrite a toxic molecule. The plant extracts were represented potent nitric oxide scavenging potential from 0.0.25to4 mg /ml. In this procedure sodium nitroprusside was used as a source for nitric oxide generation in laboratory which combines with oxygen to produce nitrite ions. Griess reagent was applied for determination of nitrite ions in present study. There was a direct positive correlation between percentages of inhibitions with plant extract concentration. Nitric oxide same as reactive oxygen species associated in inflammation processes, degenerative disease, pathological disorders and cancers (Mohamed A, 2009). The plant extract may have potential against nitric oxide generation, stop of chain of reactions and preventing of relative disease formation in live organisms.

The result revealed that the *Quercus brantii* and *Juglans regia* extract may contain compounds that capable to inhibit nitric oxide and related inflammatory disease.

Hydroxyl radicals are high reactive molecules can be formed from hydrogen peroxide in the presence of iron by Fenton reaction in live organisms. Hydroxyl radicals attack to biomolecules such as proteins, DNA, polysaccharides and lipids in cell particularly in cell membranes (Olabinri, 2010). In recent research hydroxyl radical was generated by mixing iron (II) sulphate that generate ferrous ion with hydrogen peroxide and 1-10-phenanthroline. The 1, 10-phenanthroline was carried out because phenanthroline-Fe^{2+} is a frequently applied for detected of redox reaction. The hydrogen peroxide / ferrous iron system yields hydroxyl radical by the Fenton reaction with phenanthroline- ferrous iron complex oxidizes to ferric iron (Olabinri, 2010). *Quercus brantii* and *Juglans regia* demonstrated concentration dependent scavenging potential against hydroxyl radical. This finding confirmed by Rivas-arreola study on *Quercus* species infusion (Riva-Arroeola, 2010).

5. Conclusion

The leaves extract of *Quercus brantii* and *Juglans regia* were showed good but different levels of antioxidant potential in all the models studied. The extracts hadgood ironchelating potential, nitricoxide, hydroxyl and Superoxide radical scavenging activities. *Quercus brantii* and *Juglans regia* extracts could be used in the treatment of iron-overload disordersdueto its high chelating potential at low doses. Furtherstudied of individual compounds for their in vivo antioxidant Potential is necessary.

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