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Antioxidant enzymes activity of *Ferula flabelliloba* and *Ferula diversivitata* extracts

*Ferula flabelliloba* ve *Ferula diversivitata* ekstraktlarının antioksidan enzim aktiviteleri

**Abstract:** Objective: Free radicals are generated during different reactions in cells and are potentially threats to macromolecules such as DNA and protein. Cells have established defense systems to remove these radicals. In some diseases, the systems are defective because of different damages and cells cannot remove the radicals. Plants have been used for a long time as sources of antioxidants for treatment of different diseases. *Ferula* species have also been used and investigated as a source of antioxidants in Iranian herbal medicine.

Methods: Here, we have studied the antioxidants activity of catalase, superoxide dismutase and lipid peroxidation from aqueous and ethanolic extracts of *Ferula flabelliloba* and *Ferula diversivitata* plants.

Results: Our results have shown that they have significant antioxidant activity in different parts at different stages.

Conclusion: We can conclude that these plants extracts can be used for more studies as antioxidant sources.

**Keywords:** *Ferula*, antioxidant, catalase, superoxide dismutase, lipid peroxidation

**Özet:** Amaç: Serbest radikallerin hücrelerde farklı reaksiyonlar sırasında üretilmekte ve DNA'ya da protein gibi makromoleküllere tehdit oluşturur potansiyeli bulunmaktadır. Hücreler bu radikalleri ortadan kaldırmak için savunma sistemlerini kurmuşlardır. Bazı hastalıklarda ise bu sistemler hasar görerek bozulur ve hücreler serbest radikalleri temizleyemezler. Bitkiler, farklı hastalıkların tedavisi için antioksidan kaynağı olarak uzun bir zamandır kullanılmaktadır. İran'da uygulanan bitkisel tedavilerde kullanılan *Ferula* türleri antioksidan kaynağı olarak araştırılmaktadır.

Metod: Bu çalışmada, *Ferula flabelliloba* ve *Ferula diversivitata* su ve etanol ekstraktlarından elde edilen katalaz, süperkoksit dismutaz ve lipit preoksidaz antioksidan etkileri araştırılmıştır.

Bulgular: Sonuçlarımız bu bitkilerden elde edilen katalaz, süperkoksit dismutaz ve lipit preoksidazın değişik aşamalarda belirgin antioksidan etkisi sahip olduklarını göstermiştir.

Sonuç: Sonuç olarak bu bitkilerin ekstrerelinin antioksidan kaynağı olarak çalışmalarında kullanılabileneğini söyleyebiliriz.

**Anahtar Kelimeler:** *Ferula*, antioksidan, katalaz, süperkoksit dismutaz, lipit peroksidaz

**Introduction**

Free radicals are well known as short-lived species that are highly reactive to very important macromolecules including DNA [1]. They have already been shown to contribute to numerous diseases including cancer and cardiovascular diseases [2]. To decrease these threats, cells have
established different systems, most importantly are antioxidants species and enzymes [3]. In some diseases, including cancer, these antioxidants are not available because of defects in the system already resulted due to high frequency of free radicals [4]. The harmful action of these radicals can be blocked by natural antioxidants from other sources such as plants. Several plants have been shown to possess antioxidant activity [5]. However, the search for plants containing more powerful and natural antioxidants is still increasing.

The Ferula genus that belongs to Apiceae has been shown to be a source of resins [6]. It has been used as a sedative for treatment of several disorders such as headache, arthritis, diabetes, digestive disorders and rheumatism and also the essential oils of this genus have shown antibacterial, antiviral, antifungal and anticancer properties [7]. Ferula genus revealed to possess phyto-chemicals including sesquiterpenes and coumarins. Ferula genus has almost 150 species native to central Asia and Mediterranean and nearly 30 species native to Iran [8]. Ferula flabelliloba and Ferula diversivitata are wild plants native to Iran. They have been used in traditional medicine as sedative for diarrhea and abdominal pain [7]. There are numerous studies on chemical compositions and potential of antioxidant activities of Ferula genus essential oils and extracts [9]. However, there is no report of antioxidantive enzyme activity of these two species. Here, for the first time, we have investigated the activity of catalase, superoxide dismutase (SOD) and lipid peroxidation in extracts of F. flabelliloba and F. diversivitata.

Materials and Methods

Plant materials and extract

The plants includes aerials and root parts of F. flabelliloba and F. diversivitata were collected from Binalood Mountains and Ataieh heights of Neishabour, Iran in July 2013, at two different vegetative and reproductive stages, and were immediately transferred to and deposited in physiology laboratory of Islamic Azad University of Neishabour, Iran.

The plants were identified by Dr. V. Mozaffarian (Department of Botany, Research Institute of Forests and Rangelands, Tehran, Iran). A voucher specimen of each species (F. flabelliloba; No. 1001; F. diversivitata; No. 1002) has been deposited in the Herbarium of the Department of Microbiology, Neishabour Branch, Islamic Azad University, Neishabour, Iran. The plant materials were air-dried at room temperature, protected from light, for 1 week. Then samples were freeze dried and storage in -20°C for later usage. For each assay according to its protocol were don. Also GC/MS was used to isolation and characterization of plant compounds which was reported previously (in Persian).

Chemicals

Ferrozine, Linoleic acid, Nitro Blue Tetrazolium (NBT), trichloroacetic acid (TCA), and potassium ferricyanide were purchased from Sigma Chemicals Co. (USA). Hydrogen peroxide, ethylenediaminetetraacetic acid (EDTA) and Ferric chloride were purchased from Merck (Germany). All other chemicals were of analytical grade or more pure.

Extract preparation for enzymes assay

To measure antioxidant enzymes, the preparation extract for enzyme assay preparation was performed using Sairam method [10]. Briefly, 0.5 g dried sample was grind and 5 ml extract solution (ethanol or distained water) was added and the mixture was filtered. The resulting solution was centrifuged at 15000 rpm for 15 min at 4°C. The supernatant containing enzymes was used for enzymatic assays.

Catalase activity

The activity of catalase was measured according to Pereira et al. method [11] using measuring the amount of H2O2 at 240nm. The assay was performed by adding appropriate of prepared extract for enzyme assay (10µl of 0.1gr/ml) to mixture of 1.9ml potassium phosphate as buffer (0.5mM, pH=7) and 1ml of H2O2(3mM) in 3ml final volume reaction. The absorbance was immediately measured at 25°C for 2min every 30 sec using spectrophotometer and enzyme activity was measured using following formula: \[ \Delta A/e=\text{Unit/mg fresh weight (}\Delta A=\text{absorbance changes per 2min, } e=\text{quiescent coefficient)} \]

Superoxide dismutase activity

This test was performed according to Beauchamp & Fridovich method [12]. Briefly, 100µl of 0.1M EDTA-Na, 60µL of 75µM NBT, 400µl of 13mM L-methionine and 400µl of 4mM riboflavin were added to 2ml potassium phosphate (0.05M) buffer (pH=7.5) in a dark tube. 40µl of prepared extract (0.1gr/ml) for enzyme assay was added
to achieve 3ml final reaction and tubes were immediately subjected to fluorescence light. After 15min incubation, the absorbance was measured at 560nm. The reaction without extract was used as calibrator. The enzyme activity was calculated by Beauchamp & Fridovich formula. Where OD is control absorbance, od is sample absorbance and Unit SOD is enzyme unit per tissue wet weight.

**Lipid peroxidation**

The lipid peroxidation activity was measured using Health & Packer method [9] by forming Malondialdehyde (MDA) complex from membrane lipids peroxidation by tributyltin (TBT). Briefly, 0.5 g wet leaf was grinded with trichloroacetic acid 0.1% and then centrifuged at 15000 rpm for 5min. 1ml supernatant was mixed with 3ml trichloroacetic acid 20% containing thiobarbituric acid 0.5%. The resulting solution was incubated at 90°C for 30min and the transferred to ice. Then, it was centrifuged at 15000 rpm for 5min at 4°C and absorbance was measured at 532nm. The Malondialdehyde was concentration was obtained from A=εbc formula where A is sample absorbance, ε is quiescent coefficient and c is Malondialdehyde concentration (µmol/gFw).

**Statistical analysis**

The data were analyzed by an analysis of variance (p <0.05) and the means separated by Duncan’s multiple range tests. The experimental results are expressed as means±SD. All measurements were performed as triplicate.

**Results**

**Catalase enzyme activity assay**

As shown in Figure 1, the results demonstrated that maximum activity of catalase for *F. flabelliloba* was seen in roots at reproductive stage and aerial parts at vegetative growth. However, different parts have not shown significant difference. The maximum and minimum activity of catalase for *F. diversivitata* was seen in aerial parts at reproductive stage (0.283 unit/mgWW) and aerial parts at vegetative parts (0.125unit/mgWW). There was a significant difference between parts of *F. diversivitata* species.

**Superoxide dismutase enzyme activity assay**

As shown in Figure 2, the results demonstrated that maximum activity of superoxide dismutase for *F. flabelliloba* was seen in roots at reproductive stage and aerial parts at vegetative growth. However, different parts have not shown significant difference. The maximum and minimum activity of superoxide dismutase for *F. diversivitata* was seen in aerial parts at reproductive stage (0.283 unit/mgWW) and aerial parts at vegetative parts (0.125unit/mgWW). There was a significant difference between parts of *F. diversivitata* species.

**Lipid peroxidase enzyme activity assay**

As shown in Figure 3, the results demonstrated that maximum activity of lipid peroxidase for *F. flabelliloba* was seen in roots at reproductive stage and aerial parts at vegetative growth. However, different parts have not shown significant difference. The maximum and minimum activity of lipid peroxidase for *F. diversivitata* was seen in aerial parts at reproductive stage (0.283 unit/mgWW) and aerial parts at vegetative parts (0.125unit/mgWW). There was a significant difference between parts of *F. diversivitata* species.
Superoxide dismutase activity assay

As shown in Figure 2, for *F. flabelliloba*, the maximum activity of SOD was shown in aerial parts. For *F. diversivitata*, the maximum activity was seen in aerial parts at reproductive stage (63.8 unit/mgWW) and the minimum was seen in roots at vegetative stages. In overall, these results show that the activity of superoxide dismutase in roots at reproductive stage is more than vegetative stage.

Lipid peroxidation activity assay

The data shown in Figure 3 indicates that different parts have significant effects on lipid peroxidase activity of *F. flabelliloba* and *F. diversivitata* (*p*=0.001) in Compared with each other according to the ANOVA analysis. The maximum and minimum activity for *F. flabelliloba* was seen in aerial parts (0.086µg/gWW) and roots (0.015µg/gWW), respectively. Also, the maximum and minimum activity seen for *F. diversivitata* was in aerial parts (0.026µg/gWW) and roots (0.007µg/gWW), respectively. At vegetative stage. Totally, the results show that the lipid peroxidase activity seen in aerial parts was more than roots (*p*=0.003).

Discussion

In this paper we aimed to investigate that is the extracts have any antioxidant properties? And next we compared these properties with each other. So we don’t use any positive control. Comparison of anti-oxidant effect (anti-lipid and anti-protein peroxidation) against BHT and BHQ is performed for next paper. At the other hand, only we have verified that plant extracts have anti-oxidant effect.

Free radicals are generated during several reactions in our body and are biological threats to different molecules such as DNA, lipids and proteins that are essential participants of a cell [13]. To decrease against them, the cell has evolved defense systems including antioxidant enzymes [14]. However, these systems are defective due to various damages in multiple diseases. Antioxidants such as catalase, superoxide dismutase and lipid peroxidation have been shown to be highly reactive in essential oils of different plants [15]. The essential oils from numerous plants have been widely used for treatment of diseases such as arthritis, headache and cancer [16]. To use as drugs, looking for plants essential oils containing high antioxidant activity still continued. Here, we have investigated the activity of three antioxidant enzymes from prepared extract of *F. flabelliloba* and *F. diversivitata*.

Catalase, one of the most important antioxidant enzymes, catalyzes H\textsubscript{2}O\textsubscript{2} that is formed during fatty acids oxidation [17]. To measure this enzyme activity, Pereira et al. [11] method has been used which is based on measuring the amount of H\textsubscript{2}O\textsubscript{2} at 240 nm. The results from this experiment showed that the maximum activity was seen in aerial parts at vegetative growth. For *F. diversivitata*, the maximum and maximum activity was seen in aerial parts and roots at reproductive growth. This shows that the catalase activity differs from a part to another part of plant. It has been reported that increased-activity of catalase is due to environmental stresses which implies that increasing catalase activity plays an important role in reducing hydrogen peroxide effects in plants such as wheat, rice and soybean [18]. The catalase expression has shown a significant increase when exposing leaves to UV-B, however increasing the exposure time leads to reduction of catalase expression [19]. This reduction was particularly more obvious at higher exposure for alfalfa leaves. There are some reports consistent with ours that shows role of environmental stress in regulation of catalase activity. It is apparently due to intense cellular oxidative stress followed by cell death [20].

Different researches have shown that oxidative stress toleration is strongly associated with increasing antioxidant enzymes activity in plants [21]. Researchers have demonstrated that the antioxidant enzymes including superoxide dismutase concentration increases at stress conditions such as drought and accordingly enhances resistance to oxidative stress. Also, it has been previously reported that SOD activity is increased in response to different environmental stresses such as high exposure to light and salinity [22]. Antioxidant enzyme such as SOD plays an important role in removing free radicals of cells [23]. Our study showed that SOD activity was not dependent to different parts. In overall, in aerial parts, the maximum activity was seen at reproductive stage. It seems that the plant have exposed to environmental stresses. Since the growth area of *F. diversivitata* has a low raining, the environmental stresses occur more to this plant leading to enhanced-SOD activity. Moreover, Tan et al. reported that increasing drought at reproductive stage decreases SOD activity [24]. This supports the current results showing low activity of SOD in roots at different stage for *F. flabelliloba*.

Waskiewicz et al. [25] have reported that increased concentration of Malone dialdehyde (MDA) indicates the elevated lipid peroxidation and fatty acids oxidation. Environmental stresses generate superoxide radicals which in turn cause lipid peroxidation. Drought and
loss of water causes oxidative stress and deregulation of physiologic conditions followed by oxygen free radicals formation [25]. The current study shows that lipid peroxidation activity differences in roots at different stages was significant. The maximum activity for both species was shown at reproductive stage. This may be because of early exposure to environmental stresses such as coldness and sunlight and drought. The increased environmental stress including loss of water at reproductive stage resulted to increased activity of lipid peroxidation in roots as previously reported by Sairam et al. [10].

Conclusions

Medical plants extract have been widely interested to be used for treatment of different diseases. Some of them are rich sources of antioxidant, antifungal and anticancer compounds. *Ferula* species, also, have been shown to have antioxidant activities. Here, *F. flabelliloba* and *F. diversivitata* that grow up in Eastern Iran especially Binalood Mountains, have shown significant antioxidant activity (p<0.05). Accordingly, they maybe used as sources of antioxidants for treatment of different diseases. We suggest that Comparison of anti-oxidant effect (anti-lipid and anti-protein peroxidation) against BHT (Butylated hydroxytoluene, also known as butylhydroxytoluene) and BHQ (butylhydroquinone, also known as tertiary butylhydroquinone; TBHQ) as standard anti-oxidants should be performed.

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