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Evaluation of the Total Antioxidant Capacity of Bitter and Sweet Varieties of *Ferula assa-foetida* and *Bunium persicum*

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Abstract

Background: Due to low information about total antioxidant capacity of three species of plants native to Ilam province which are used in the ethnobotanical knowledge of this region, This study was drafting to evaluation the antioxidant ability of bitter and sweet varieties of *Ferula assa-foetida* and *Bunium persicum* with therapeutic potential on gynecological diseases.

Methods: The methanolic extracts of two different variants of *F. assa-foetida* and *B. persicum* gum-resin were prepared and then antioxidant effects were evaluated by ferric reducing-antioxidant power assay.

Results: Our results showed that methanolic extracts of *B. persicum* gum-resin could significantly revealed antioxidant effect in comparison to two different variants of *F. assa-foetida* ($P < 0.05$). While antioxidant capacity between bitter and sweet varieties of *F. assa-foetida* were not statistically significant.

Conclusion: Our results showed that both *B. persicum* and the bitter and sweet varieties of *F. assa-foetida* native to Ilam province, located in west of Iran, could have medicinal therapeutic effects relatively through direct oxidation prevention.

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Keywords:

Total antioxidant capacity;
Ilam; *Bunium persicum*;
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Introduction

Produced free radicals in the body cause to chronic and dangerous diseases including atherosclerosis, ischemia, arthritis, cancer, and chronic fatigue [1-3]. So, recently there has been an increase of interest finding the therapeutic medicinal plants as antioxidants in decreasing free radical induced tissue injuries. Moreover, well known and traditionally used natural plant based antioxidants have been considered in the search for innovative antioxidants. But there is still the request in the scientific and industrial societies to find more evidence concerning the antioxidant potential of additional plant species. Traditional herbs contain various metabolites, which have confirmed a varied range of pharmacologic activities. Antioxidant's efficacy of some herbs have been revealed to play a protective character in the body against mentioned diseases [2-4]. Traditional medicine knowledge is based on finding local and natural resources and ingredients to improve lifestyles. Many people in the west Asian region who consult with pharmacognosists, herbalists, and ethnobotanists are applying herbal traditional therapies. *Ferula assa-foetida* L. (Apiaceae) is one of the most important among the species of *Ferula* dispersed in Iran. *F. assa-foetida* is a medicinal, rangeland, and industrial plant that the sap or gum is obtained by paring the roots of the plant which has a bitter sulfur odor and smell similar to the rotten garlic [5, 6]. Based on the stinking assa species, two types of bitter and sweet gum resin are harvested. Despite the apparent differences in the species of plants and harvested gums, both species belong to the *F. assa-foetida* [7]. In traditional medicine, oleo-gum resin which is named asa-foetida or Anghoze in Persian has anticonvulsant, sedative, carminative, antispasmodic digestive, expectorant, laxative, analgesic, anthelmintic, antiseptic, remedy for abdominal and kidney pain, and anti-muscle cramps, effect on blood pressure, and improve mental performance and increases learning and memory [5, 7]. *B. persica* is a medicinal plant from the Apiaceae family. The name in Persian included Syahzirah, Kalazirah, and Zirah kahi. Medicinal plants native to countries have been a continuous source of drugs and recently, scientists have emphasized finding new therapeutic agents from these plants to reduce the dose and frequency of the prescribed chemical drugs [8, 9]. *B. persicum* has three principal active ingredients contains different monoterpenes, sesquiterpenes and phenyl propenes [8, 10].

Studies are encouraged creating new natural base medications to reduce clinical symptoms and improve quality of life, particularly in industrialized countries. As a result, the current investigation was carried out to evaluation the in vitro antioxidant impact of *Elwendia persica* and *F. assa-foetida* L.

Methods

Plant preparation

In April 2022, medicinal plants of *Ferula assa-foetida* are prepared from Dehloran and Ilam cities located in Ilam province, west of Iran. The plant was identified and approved using the morphological keys mentioned in the book of plant flora of Ilam province in BMPRC of IUMS, Iran. Collected plants was washed, cleaned, dried under shade, subsequently, the dried plant was powdered by a commercial plant mixer and used for antioxidant evaluations. The characteristics of the mentioned medicinal plant used in this study are specified in Table 1.

Preparation of plant extract

After drying the collected plants, one gram of homogenized dry powder of the selected plant was added to 100 mL of methanol solution and shaken for 48 hours. The resulting solution was then poured into a tube and centrifuged at 6000 rpm for 10 minutes. The obtained samples were filtrated through Whatman filter No. 1. Then the methanol was allowed to evaporate on dry oven to dry at 35 °C and then the concentrated extract was stored at 4 °C [11, 12].

FRAP (ferric reducing-antioxidant power) assay

First, 5µl of the prepared plant solution was added to each well, and then 250 µL of the working solution was added to each well containing the plant solution. The microplate was then incubated for 30 minutes at 35 to 50 °C and finally read at 570 nm with the Elizar Reader.

Preparation the working solution

2.2 mL of R2b solution was added to the R2a tube, vortexed until complete dissolving, and finally R2 solution was obtained. Then R2 solution was mixed, vortexed 5 times, and added to R1 solution in a ratio of 1: 1. The obtained solution was the working solution of an antioxidant kit.

Preparation of standard solution

Standard solution with different concentrations of 0, 0.2, 0.4, 0.6, 0.8, and 1 was also prepared.

Evaluation of antioxidant effects

Five µL of the prepared plant solution was added and then 250 µL of the prepared working solution was added. The microplate was then incubated for 30 minutes at 35 to 50 °C and finally read at 570 nm with the Elisa reader.

Statistical analysis

All statistical analyses of biochemical estimations were performed using the One-Way ANOVA using Graph Pad Prism (5.04). A p-value of less than 0.05 was deemed a significant difference in all studies. analysis of variance (ANOVA), and logistic regression were used for data analysis.

Results

The results of the total antioxidant capacity for the methanol extract of the plants of this study are given in Table 2. Based on this, the total antioxidant capacity of *Bunium persicum*, bitter stinking assa and sweet stinking assa with a total antioxidant value of 1.75 mmol Fe²⁺/L, 1.18 mmol Fe²⁺/L and 1.09 mmol Fe²⁺/L, are respectively (P<0.05). While Sweet stinking assa collected from Ilam city had low antioxidant activity (1.09 mmol Fe²⁺/L) comparing to the Bitter stinking assa (1.18 mmol Fe²⁺/L) collected from Dehloran city but these differences were not statistically significant (P>0.05).

Persian Name of Plants	Scientific Name	Family	Region of Collected the Plants	Geographical Coordinates
Anghouzeh talkh (Bitter stinking assa)	<i>Ferula assa-foetida</i>	Apiaceae	Dehloran	32° 41' 28" North, 47° 15' 58" East
Anghouzeh Shirin (Sweet stinking assa)	<i>Ferula assa-foetida</i>	Apiaceae	Ilam	32° 41' 28" North, 47° 15' 58" East
Zireh kuhi	<i>Bunium persicum</i>	Apiaceae	Ilam	32° 41' 28" North, 47° 15' 58" East

Table 1: The medicinal plant used details of the present study

Total antioxidant capacity	Persian Name of Plant
<i>Ferula assa-foetida</i> Anghouzeh talkh (Bitter stinking assa)	1.18 mmol Fe ²⁺ /L
<i>Ferula assa-foetida</i> Anghouzeh shirin (Sweet stinking assa)	1.09 mmol Fe ²⁺ /L
<i>Bunium persicum</i> Zireh kuhi	1.75 mmol Fe ²⁺ /L

Table 2: Total antioxidant activity of methanolic extracts of bitter and sweet *Ferula assa-foetida* and *Bunium persicum*

Discussion

Free radicals have destructive effects on the cells of the body and by spreading, they destroy all tissues. Antioxidants are chemicals that prevent the spread of free radicals and fight them. Free radicals are unstable atoms that can damage cells and cause disease and aging [13-17]. In fact, free radicals are toxic products of oxygen metabolism. Irreversible damage leads to changes in cell function or cell death. These destructive factors are the cause of many diseases [18-25].

So for finding natural based anti-oxidant agents, in the present study, 3 medicinal plants were collected and analyzed for their antioxidant activity. Followed oxidative stress conditions, reactive nitrogen species and reactive oxygen species production is increased, subsequently cause alteration of membrane lipids, nucleic acids and proteins. Oxidative damage of produced biomolecules related to age increasing and pathological events (atherosclerosis, degenerative disorders and ischemia reperfusion injury) [25, 26]. Some medicinal plants contain high amounts of

antioxidants, the consumption of these plants can be effective in human health [27-33], and their antioxidant effect is due to the presence of effective substances [34-39].

In this laboratory study, the ferric reducing antioxidant power (FRAP) assay was used to evaluation the antioxidant activity of three collected plants native to Iran. This method is based on the capacity of the detection the formed ferrous ion (Fe²⁺) which can be examined by absorbance capacity at 570 nm. So, *Bunium persicum* showed the high antioxidant activity in comparison to the two species of *Ferula assa-foetida* (P<0.05). While Sweet stinking assa had low antioxidant activity (1.09 mmol Fe²⁺/L) comparing to the Bitter stinking assa (1.18 mmol Fe²⁺/L). Previous research have shown that herbs contain composition, which are responsible for antioxidant efficacy. The isolated and confirmed resin of *F. assa-foetida* fraction contains ferulic acid esters, free ferulic acid, umbelliferone, and coumarin derivatives such as foetidin and farnesiferoles [40-42]. In present study the antioxidant activity of collected bitter and sweet form of *F. assa-foetida* resin from Ilam province in comparison to *B. persicum* has been approved by ferric reducing antioxidant power (FRAP) assay.

has the main principal active ingredient in the methanolic extract called γ - terpinene which seems to be responsible for their antioxidant activity. Additionally, black cumin seed contains cuminal, p-cymene, limonene, and 1,4-p-menthadien-7-al that contribute to anti-oxidative activity of extract [43,44].

Coumarins detected in *F. assa-foetida* resin have been associated with beneficial effects on human health, such as reducing the risk of different cancers, diabetes, cardiovascular and central nervous system diseases. These effects relate to the radical scavenging effect, due to their antioxidant activities, beside anti-inflammatory properties and interaction with several enzymes which confirmed in present study. In other hands, Thymoquinone (TQ) is one of the main constituents derived from *B. persicum* which has different pharmacological properties such as antioxidant activities and anti-inflammatory. *B. persicum* showed the more anti-oxidative effect in comparison to the two species of selected *F. assa-foetida* [43-45].

B. persicum as a plant of the Apiaceae family cultivates in different regions of Asia such as Iran. Based on previous studies, this plant showed significant medicinal, antimicrobial and antioxidant properties. In traditional medicine of Iranian ethnobotany, it was used to treatment or improvement the digestive and urinary disorders, diabetes, and obesity with antimicrobial, antioxidant, anti-inflammatory, and analgesic properties. *B. persicum* alcoholic extract contains high levels of oxygenated monoterpenes, especially γ -

Terpinene which has high antimicrobial and antioxidant effects. *B. persicum* is widely used in people's diet as an additive agent that there is no most important concern about the toxic properties of this plant [9, 43]. The use of medicinal plants is recommended to increase the antioxidant capacity [46-52].

In conclusion, our results show that both varieties of *F. assa-foetida* and *B. persicum* which grows in Ilam province, located in west of Iran, prevent and also decrease the rate of oxidation which was evaluated by ferric reducing antioxidant power (FRAP) assay due to the active pharmaceutical ingredient components. However, statistically significant effects were not found for the bitter and sweet variant of *F. assa-foetida*, possibly due to the same components in both varieties. Considering the high prevalence of diseases caused by oxidative stress as well as the good antioxidant effect of medicinal plants, it is recommended to use their antioxidant capacity in pharmacological clinical studies.

Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

Zohre Eftekhari, Dhiya Altememy: Data analysis
Roua Abulkassim, Noora M. Hameed: Monitoring of research
Zohre Eftekhari, Indrajit Patra, Thulfeqar Ahmed Hamza, Ayat Hussein Adhab, Safa K. Hachim, Roua Abulkassim: Drafting
Ayat Hussein Adhab, Safa K. Hachim, Roua Abulkassim, Noora M. Hameed: Manuscript scanning
Zohre Eftekhari, Dhiya Altememy, Indrajit Patra, Thulfeqar Ahmed Hamza, Ayat Hussein Adhab, Safa K. Hachim, Roua Abulkassim, Noora M. Hameed: Data evaluation.

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