

## Antioxidant effects of hydroalcoholic and polyphenolic extracts of *Peucedanum pastinacifolium* Boiss. & Hausskn.

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### Abstract

Antioxidant activity of *Peucedanum pastinacifolium* Boiss. & Hausskn aerial part hydroalcoholic extract (HAE) and polyphenolic extract (PPE) as well as their total phenolic and flavonoid contents were studied. Phenolic and flavonoid contents were respectively estimated as gallic acid and quercetin equivalents. The *in vitro* antioxidant activity of two extracts of *P. pastinacifolium* were evaluated by radical scavenging of 1,1-diphenyl-2-picryl hydrazyl radical (DPPH), chelating activity on ferrous ions, or ferric reducing antioxidant power (FRAP) assay. In addition, the *in vivo* antioxidant activity of hydroalcoholic extract was measured by FRAP assay. Total phenolic contents of PPE and HAE were  $117.1 \pm 6.2$  and  $44.3 \pm 1.7$  mg/g, respectively. Total flavonoid content of PPE ( $43.4 \pm 2.1$  mg/g) was found to be higher than that of HAE ( $8.0 \pm 1.5$  mg/g). In DPPH radical scavenging assay, HAE and PPE showed fifty percent inhibitory concentration (IC<sub>50</sub>) values of  $469.4 \pm 9.3$  µg/mL and  $128.2 \pm 5.5$  µg/mL, respectively. Iron chelating activity assays indicated IC<sub>50</sub> values of  $657.5 \pm 13.2$  µg/mL and  $735.4 \pm 16.1$  µg/mL for HAE and PPE as opposed to ethylenediamine tetra-acetic acid (EDTA) being  $16.5 \pm 0.8$  µg/mL. PPE exhibited greater FRAP value ( $154.0 \pm 1.8$  µM) as compared with that of HAE being  $69.3 \pm 1.4$  µM. In animal study, HAE showed a significant ( $p < 0.05$ ) increase in FRAP level when compared with that of control group. Our results showed that *P. pastinacifolium* possess antioxidant properties which most likely are exerted through free radical scavenging, chelating activity, and reducing power.

**Keywords:** *Peucedanum pastinacifolium*; Antioxidant; Reducing power; Free radicals; Chelating activity

### INTRODUCTION

Studies in the past two decades, show that free radicals play a key role in the pathogenesis of many diseases, such as atherosclerosis, ischemic heart disease, arthritis, cancer, Alzheimer, Parkinson, diabetes and aging process (1). Antioxidants neutralize the oxidative stress by inhibiting free radicals and thus preventing tissue damages (2). Two types of antioxidants are defined as enzymatic (metabolic) and non-enzymatic (nutrient). Substances such as ascorbic acid (vitamin C), tocopherol (vitamin E), flavonoids, lipoic acid and carotenoids act as nutrient antioxidants (3). Nowadays, pharmaceutical industries are paying special attention to natural antioxidants because they are safer than synthetic antioxidants (4). The

natural phytochemical compounds such as effective antioxidants play an important role in prevention and treatment of many human diseases. In recent years, it has been proven that phytochemical antioxidants such as secondary plant metabolites and plant pigments possess an inhibitory effect on free radicals, lipoperoxidation and oxidative stress (5).

*Peucedanum* is an important genus of Umbelliferae family and encompasses more than 120 species (6). Several species of *Peucedanum* genus such as *P. japonicum*, *P. graveolens* (dill), *P. oreoselinum*, *P. palimbioides* and *P. longifolium* have shown antioxidant activities (7-10). *Peucedanum pastinacifolium* Boiss. & Hausskn, another species of this genus grows

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in western and central regions of Iran. This plant is called "Alafe-tofangchi" locally and traditionally is used as a lipid-lowering vegetable. One report has shown that *P. pastinacifolium* could decrease blood lipids levels in streptozotocin-induced diabetic rats (11). In another study, ethanolic extract of *P. passtinacifolium* showed lipid-lowering activity in hypercholesterolemic rats (12). Phytochemical investigation on acetonetic extract of *P. passtinacifolium* has led to isolation of one phenyl propanoid and some furanocoumarin compounds. Moreover, forty-nine volatile compounds identified from essential oil of *P. passtinacifolium* (13).

In the present study, antioxidant activity of *P. pastinacifolium* hydroalcoholic extract (HAE) and polyphenolic extract (PPE) as well as their total phenolic and flavonoid contents were studied for the first time.

## MATERIALS AND METHODS

### Chemicals and reagents

Gallic acid, ascorbic acid, butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), ferrozine, quercetin, Folin-Ciocalteu reagent and 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) were purchased from Sigma chemicals Co, (USA). Ethylenediamine tetraacetic acid (EDTA), ferric chloride, ferrous sulfate, acetic acid, sodium acetate, hydrochloric acid was procured from Merck industry (Germany). All other chemicals were of analytical grade.

### Plant material

The aerial parts of *P. pastinacifolium* were collected in May 2014 from the Soffeh mountains located in the west of city of Isfahan (Iran) at an altitude of 1500 m. The plants were identified by a botanist at Department of Botany at Isfahan University (Isfahan, Iran). A voucher specimen of the plant (ID No. 1146) has deposited in the herbarium of School of Pharmacy and Pharmaceutical Sciences of Isfahan University of Medical Sciences (Isfahan, Iran)

### Extraction procedures

HAE: Collected plant materials were air-dried under shade, ground to a fine powder,

extracted with percolation method using 75% ethanol. The extract was then filtered and dried in a freeze dryer to produce dried powder (yield, 14.4%).

PPE: Polyphenolic extraction was performed according to the method described by Seddik *et al* (14). Briefly, 100 g air-dried powder was extracted 3 times with ethanol 80% and 3 time with ethanol 50%. The extracts were added together, filtered and evaporated by rotary evaporator. The aqueous solution was extracted 3 times with n-hexane, 3 times with chloroform, and 5 times with ethyl acetate. The ethyl acetate phase was collected and then evaporated with rotary evaporator (yield, 1.0%).

### Animals

Male Wistar rats weighing 230-250 g were kept under standard environmental conditions of 25-30 °C and light/dark cycle 12 h/12 h and allowed free access to water and feed *ad libitum*. All animal experiments were approved by the Ethics Committee of Isfahan University of Medical Science and performed in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals.

### Determination of total phenolic contents

Folin-Ciocalteu method was used for determination of total phenolic contents (15). The extracts (20 µL, 2.5 mg/mL) were added to 1.58 mL distilled water and 100 µL Folin-Ciocalteu reagent. The solution was vortexed for 2 min and sodium bicarbonate (300 µL, 200 mg/mL) was added. The samples were incubated for 2 h at room temperature and the absorbances were measured spectrophotometrically at 765 nm. Results were expressed as milligram gallic acid equivalent per gram of the extract.

### Determination of total flavonoid contents

Total flavonoids contents were estimated with aluminum chloride (AlCl<sub>3</sub>) colorimetric method (16). Samples were prepared at concentrations of 100 and 250 µg/mL. 1 mL of sample solution was added to 4 mL distilled water and then mixed with 0.3 mL NaNO<sub>2</sub> (5%) in a 10 mL volumetric flask. Five min

later, 0.3 mL of  $\text{AlCl}_3$  (10%) was added and mixed with the solutions. Afterward, 2 mL of NaOH (1 M) was added to flask and made up to 10 mL with distilled water. Samples were left at room temperature in the dark for 10 min and absorbance was determined at 510 nm using a UV-Visible spectrophotometer. Quercetin solutions in the range of 0-100  $\mu\text{g/mL}$  were used as standards.

### ***In vitro* antioxidant activity**

#### *1,1-diphenyl-2-picryl hydrazyl radical scavenging activity*

Radical scavenging activity of the extracts was determined using DPPH radical scavenging method (17). 100  $\mu\text{L}$  of HAE (0-2000  $\mu\text{g/mL}$ ) or PPE (0-1000  $\mu\text{g/mL}$ ) were added to 1 mL DPPH (60  $\mu\text{M}$ ) and mixed vigorously. The mixture was left to stand for 10 min in the dark at room temperature and the absorbance was measured at 517 nm by a UV-Visible spectrophotometer. Ethanol (1 mL) and extracts (100  $\mu\text{L}$ ) were used as the blank, and ascorbic acid, gallic acid and butylated hydroxytoluene (BHT) were used as standards. The DPPH radical scavenging activity of the extracts was calculated using the following equation:

$$\% \text{Inhibition} = [(A_0 - A_1) / A_0] \times 100 \quad (1)$$

where,  $A_0$  is the absorbance of the (DPPH + ethanol) and  $A_1$  is the absorbance of (DPPH + samples).  $\text{IC}_{50}$  value is the concentration of extracts provided 50% inhibition of DPPH radicals.

#### *Chelating activity on ferrous ions*

In this experiment, 1 mL of either HAE or PPE at different concentrations (0 – 3000  $\mu\text{g/mL}$ ) were mixed with 0.05 mL of  $\text{FeCl}_2$  (2 mM). The reaction was started by the addition of 0.2 mL of ferrozine (5 mM) and the mixture vortexed vigorously and allowed to stand at room temperature for 10 min. Absorbance of the solution was measured at 562 nm against the blank using a UV-Visible spectrophotometer. EDTA (0 – 100  $\mu\text{g/mL}$ ) was used as the standard and the blank containing water,  $\text{FeCl}_2$  and samples (18). The percentage of chelating activity of ferrous ions was calculated by the formula:

$$\% \text{Chelating activity} = [(A_0 - A_1) / A_0] \times 100 \quad (2)$$

where,  $A_0$  is the absorbance of control ( $\text{FeCl}_2$  + Ferrozine + water) and  $A_1$  is the absorbance of samples.

#### *Ferric reducing antioxidant power assay*

The ferric reducing antioxidant power (FRAP) of the extracts was determined according to the procedure described by Benzie and Strain with some modifications (19). The FRAP reagent was prepared by mixing 300 mM of acetate buffer (PH = 3.6), 20 mM of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  solution and 10 mM of 2, 4, 6-tripyridyl-*s*-triazine (TPTZ) in 40 mM of HCl at ratio of 10:1:1 (v/v), respectively. 900  $\mu\text{L}$  of fresh solution of FRAP was added to test tube and warmed at 37 °C for 4 min. Afterwards, 30  $\mu\text{L}$  of either HAE or PPE (0-1000  $\mu\text{g/mL}$ ) were added to the mixture and incubated at 37 °C for 10 min. The absorbance was measured at 593 nm spectrophotometrically. Gallic acid, ascorbic acid and BHT were used as the positive control and the blank containing sample and solvent. The solution of  $\text{FeSO}_4$  (0-1000  $\mu\text{M}$ ) was used as the standard and the results were expressed as  $\mu\text{M}$  ferrous equivalent.

#### *In vivo antioxidant activity*

In this examination, FRAP assay was used to measure the total antioxidant activity of plasma. Twenty four rats were divided in four groups of six animals each. Group 1 served as the control and gavaged with 500  $\mu\text{L}$  of distilled water. Group 2 and group 3 were gavaged with 250 and 500 mg/kg of the HAE, respectively. Group 4 received a single daily dose of 250 mg/kg ascorbic acid (as the standard) (20). After 1, 3 and 7 days, blood samples (1 mL) were collected from retro-orbital venous plexus of the rats into heparinized tubes and centrifuged at 3000 rpm for 10 min. Afterwards, 30  $\mu\text{L}$  of plasma was used for FRAP assay. The results are expressed as  $\mu\text{M}$  ferrous equivalent.

#### *Statistical analysis*

The experiments were carried out with three to nine replicated measurements. The data were expressed as mean  $\pm$  SD and analyzed using one way analysis of variance (ANOVA,  $P < 0.05$ ). The mean results of *in vitro* tests were further assessed by Duncan test.

Dunnet-t-test was used for comparison with control group in the *in vivo* test. IC<sub>50</sub> values were calculated from the log concentration of the extract plotted against the inhibition percentage.

## RESULTS

### Total phenolic and flavonoid contents

The amount of total phenolic contents was determined by Folin-Ciocalteu method. Gallic acid was used as the standard and the total phenols were expressed as mg/g gallic acid equivalent using the standard curve equation:

$$y = 0.0007X + 0.0066, r^2 = 0.9981 \quad (3)$$

Total phenolic content of PPE and HAE were  $117.1 \pm 6.2$  and  $44.3 \pm 1.7$  mg/g, respectively. As mentioned in earlier section, quercetin was used as the standard and total flavonoids were expressed as mg/g quercetin equivalent using calibration curve equation:

$$y = 0.0007X + 0.0024, r^2 = 0.9979 \quad (4)$$

The total phenolic content of PPE ( $43.4 \pm 2.1$  mg/g) was higher than that of HAE ( $8.0 \pm 1.5$  mg/g).

### In vitro antioxidant activity

#### 1,1-diphenyl-2-picryl hydrazyl radical scavenging activity

The DPPH radical scavenging of either extract of *P. pastinacifolium* and standards were determined at different concentrations. As it is evident from Fig. 1, DPPH radical scavenging activity of the extracts was increased by increasing the extract concentrations.

Also PPE indicated higher scavenging activity than HAE almost in all tested concentrations.

The IC<sub>50</sub> values of PPE, HAE, gallic acid, ascorbic acid and BHT as standards are shown in Table 1.

#### Chelating activity on ferrous ions

The chelating activity on ferrous ions of the extracts increased with an increase in concentrations of the extract (Fig. 2). HAE, however, illustrated higher activity as opposed to PPE is indicated by lower IC<sub>50</sub> value for HAE (Table 1). IC<sub>50</sub> values of all tested compounds for iron chelating activity are listed in Table 1.

Antioxidant	DPPH free radical scavenging IC <sub>50</sub> (µg/mL)	Fe <sup>2+</sup> chelating activity IC <sub>50</sub> (µg/mL)	FRAP assay (µmol/L)
Poly phenolic extract	128.2 ± 5.5	735.4 ± 16.1	154.0 ± 1.8
Hydro alcoholic extract	469.4 ± 9.3	657.5 ± 13.2	69.3 ± 1.4
Gallic acid	9.2 ± 0.3	-	893.4 ± 12.0
Ascorbic acid	18.0 ± 0.7	-	837.9 ± 9.1
BHT	20.4 ± 1.0	-	291.7 ± 4.5
EDTA	-	16.5 ± 0.8	-

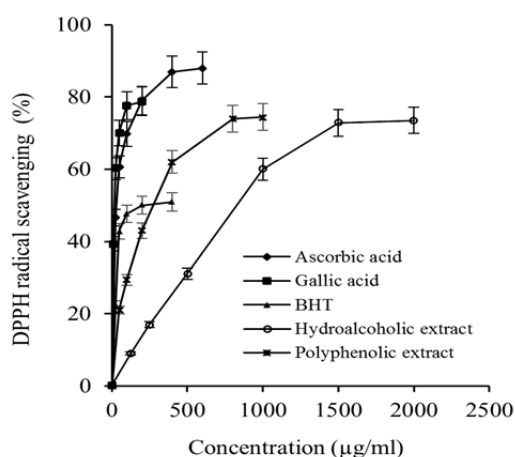


Fig. 1. DPPH radical scavenging activity of the extracts of *P. pastinacifolium* (n = 3).

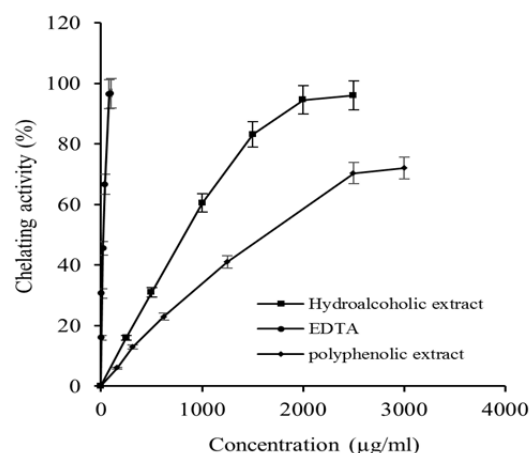


Fig. 2. Chelating activity on ferrous ions of extracts of *Peucedanum pastinacifolium*. (n = 3).

**Ferric reducing antioxidant power assay**

This assay is based on the ability of antioxidant substance to reduce ferric ions and the measurement of colored complex of TPTZ-ferrous ions at 593 nm (19). Ferric reducing ability of the extracts is shown in Fig. 3. The FRAP values of all tested compounds were increased by increasing their concentrations.

The FRAP values of PPE, HAE along with those of tested compounds are given in table 1. PPE showed grater FRAP as compared with HAE.

**In vivo antioxidant activity**

The FRAP values of the rat plasma after administration of different concentrations of HAE of *P. pastinacifolium* and ascorbic acid (as standard) over a period of 7 days are shown in Fig. 4. In groups 2 and 3 who received respectively 250 and 500 mg/kg of the HAE, a significant increase in FRAP values on day one, day 3 and day 7, in comparison with control group, was observed. However, this parameter did not changed significantly amongst groups received different doses of the extracts.

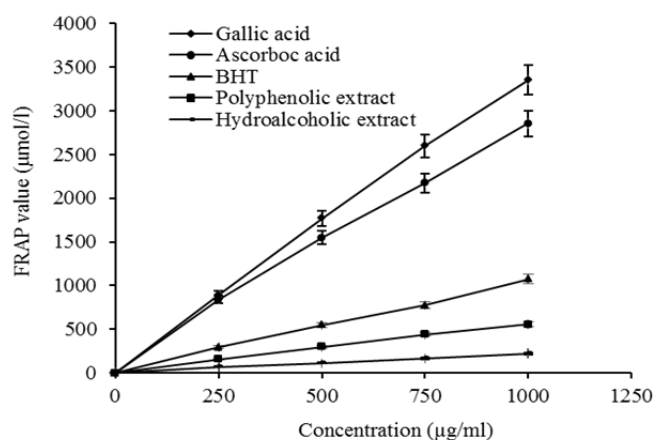


Fig. 3. Ferric reducing ability of extracts of *Peucedanum pastinacifolium*. (n = 3)

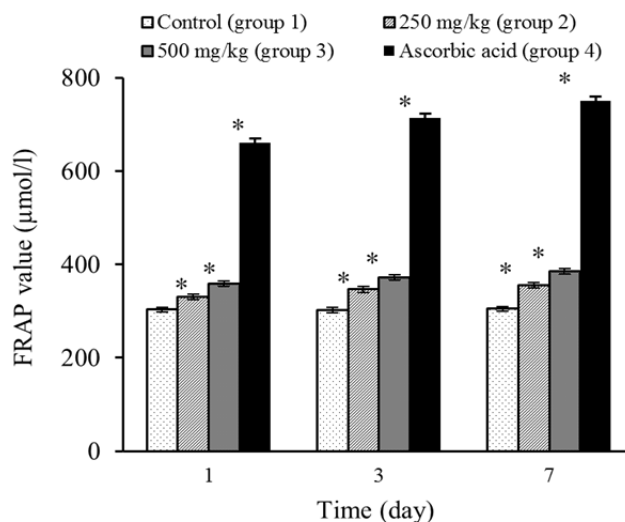


Fig. 4. Ferric reducing ability of plasma (FRAP) in rats after administration of hydroalcoholic extract of *Peucedanum pastinacifolium* (250 and 500 mg/kg) and ascorbic acid (250 mg/kg) as standard. (n = 6). \*  $P < 0.05$  compared with control.

## DISCUSSION

In the present study, both extracts showed free radical scavenging activity. Scavenging activity of extracts and standards decreased in the order of gallic acid > ascorbic acid > BHT > PPE > HAE (Table. 1). DPPH radical scavenging activity of PPE was greater than some other plants from Umbelliferae family such as *Prangos ferulacea* (L.) Lindl., *Chaerophyllum macropodium* Boiss. and *Heracleum persicum* Desf. Moreover, HAE of *P. pastinacifolium* indicated higher scavenging activity than *Chaerophyllum macropodium* Boiss (21). Phytochemical investigation showed presence of bergapten in acetonetic extract of *P. pastinacifolium* (13) which is able to scavenge DPPH free radical due to its phenolic ring (22). Phenolic compounds act as free radical acceptors and chain breaker. They interfere with the oxidation of lipids and other molecules by rapid donation of an electron or hydrogen atom to free radicals (23). Therefore, phenolic compounds such as bergapten could be responsible for scavenging activity of *P. pastinacifolium*.

It has been proposed that the antioxidant properties of phenolic compounds can be mediated by chelating trace metals involved in free radical production. Our results showed iron chelating activity of extracts of *P. pastinacifolium*. Iron chelating activity of extracts decreased in the order of HAE > PPE (Fig. 2). Ebrahimzadeh and *et al.* reported the chelating ability of *Laser trilobum* L from Umbelliferae family (24). Phytochemical analysis demonstrated the presence of methyl eugenol and eugenol in essential oil of *P. pastinacifolium* (13). Wannes and *et al.* attributed iron chelating activity of flowers of myrtle to methyl eugenol and eugenol in its essential oil (25). Thus, these two nonpolar dihydroxylated compounds could be responsible for chelating activity of HAE of *P. pastinacifolium*.

Ferric reducing ability of extracts and standards are demonstrated in Table. 1. The extracts showed the low reducing activity in comparison to gallic acid, ascorbic acid and BHT as standards. Gallic acid possessed the highest FRAPS value, followed by ascorbic

acid, BHT, PPE and lastly HAE. In two separate studies, antioxidant properties of three species of *ferula* and *Eryngium palmatum* (Umbelliferae) were investigated (26,27). Ferric reducing ability of PPE was higher than *Ferula Szowitsiana*, *Ferula hirtella* and *Ferula oopoda*. Both extracts showed better reducing activity in comparison with *Eryngium palmatum*.

The reducing activity of polyphenol molecules depends on the number of free hydroxyl groups (23). Phenolic OH-groups of flavonoids and other phenolic compounds could be responsible for better ferric reducing activity of PPE.

In the current study, total antioxidant capacities of plasma of Wistar rats were determined after HAE administration. For this purpose, ferric reducing ability of plasma was measured. As Fig. 4 indicates, the FRAP values of HAE (groups 2 and 3) were lower than ascorbic acid as the standard. Ferric reducing abilities of rat plasma of groups 2 and 3 were significantly higher than those of controls ( $p < 0.05$ ). Amongst HAE-treated rats, the Maximum ferric reducing activity of the HAE was attained after 7 days of administration. This significant increase in FRAP level displays the presence of bioactive antioxidant molecules in HAE.

## CONCLUSION

In this study, HAE as well as PPE of *P. pastinacifolium* exhibited a noticeable antioxidant effects though was not considerable to standards of gallic acid, ascorbic acid and BHT. Except iron chelating activity, the PPE showed greater antioxidant activity compared to HAE in all tests. The extracts indicated a good antioxidant activity at higher concentrations. Thus, further studies are warranted on the toxicity and isolation of antioxidant compounds of *P. pastinacifolium*.

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