



## **Antioxidant Activity, Total Phenolic and Flavonoid Contents of *Petroselinum crispum* Mill.**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author MMES designed the study, wrote the protocol and revised the manuscript in collaboration with authors NHM and IAI. Authors HAH and BSAAW managed the analyses of the study, managed the literature searches, performed the statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Background:** *Petroselinum crispum* is a common vegetable or spice in Egypt and worldwide. It possess many pharmacological and medicinal properties.

**Aims:** In the current research, the total phenolic and flavonoid contents as well as the antioxidant activities of *P. crispum* methanolic extract and its fractions were evaluated.

**Methodology:** The total phenolic content was estimated by Folin-Ciocalteu method and total flavonoid content was tested by aluminum chloride assay. The antioxidant activity was evaluated by 1, 1- diphenyl-2- picryl hydrazyl radical (DPPH) assay, 2, 2'-azino- bis (3-ethylbenzthiazoline-6-sulphonic acid) assay (ABTS), and total antioxidant capacity assay.

**Results:** The ethyl acetate fraction derived from the methanolic extract exhibited the highest total phenolic content (121.95±2.15, mg GAE/ g extract) and total flavonoids content (106.45±2.18 mg rutin equivalent / g extract). Furthermore, the ethyl acetate fraction demonstrated the more potent

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DPPH activity ( $Sc_{50}$  = 44.51±2.06 µg/ml), ABTS activity (69.82±3.07 mmTrolox<sup>®</sup> eq. / 10 g extract) and total antioxidant capacity (166.83±1.96 mg of ascorbic acid eq. / g extract).

**Conclusion:** The results of the current study showed that *P. crispum* contains an important amount of phenolic compounds and higher antioxidant activities, which serve as a good source of food ingredients and dietary supplements.

**Keywords:** *Petroselinum crispum*; antioxidant properties; phenolics; DPPH assay.

## 1. INTRODUCTION

Reactive oxygen species (ROS) are free radicals produced by living organisms as a result of normal cellular metabolism, such as superoxide anion ( $O_2^-$ ), hydroxyl radical ( $\cdot OH$ ), and hydrogen peroxide ( $H_2O_2$ ) [1]. The excessive concentrations of ROS in the human body can produce adverse modifications to the cell components (e.g., proteins, lipids, amino acids, and DNA), it can cause oxidative stress. Oxidative stress is a condition whereby the body cannot counter-attack the production of free radicals, leading to cell injury and death [2,3]. Oxidative stress has been linked to many diseases, including cancer, atherosclerosis, hypertension, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, Alzheimer's disease, and asthma [1,4,5]. Antioxidants are exogenous or endogenous molecules that protect body cells against the harmful effects of ROS by scavenging the free radicals to increase the anti-oxidative defense [6]. Antioxidants can be classified into two major types based on their source, i.e., natural and synthetic antioxidants. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butylhydroxyquinone (TBHQ), and propyl gallate (PG) are used in food industry but they are associated with some adverse effects on health [2]. Nowadays, the use of natural antioxidants such as vitamins, ascorbic acid, enzymes (catalase, glutathione peroxidase and superoxide dismutase), and various phytochemicals have increased substantially worldwide [7]. This is due to their ability to reduce the risk of various chronic diseases and safe for human health [8]. Therefore, the consumption of fruits and vegetables can prevent negative effect of oxidative stress, because they contain phenolic and flavonoid compounds which have antioxidant properties [9].

*Petroselinum crispum* Mill. (Parsley) is one of *Petroselinum* species, and belongs to *Apiaceae* family. *P. crispum* is widely distributed in European, Mediterranean and Asian countries

[10]. In Egypt *P. crispum* is widely used as an essential spice and vegetable in daily meals and salad ingredients due to its medicinal and pharmacological benefits. *P. crispum* has been used in Arab Traditional Medicine as laxative, diuretic and antiurolithiatic agents. The leaves are used as hot application against inflammatory condition, mastitis and haematomata [11]. The seeds and roots of the plant have been used to treat numerous digestive problems including diarrhoea, ulcer, flatulence and colic pain [12]. *P. crispum* is highly seasonal in nature and it is considered as a very rich source of vitamins C and E,  $\beta$ -carotene, thiamin, riboflavin and organic minerals [13]. *P. crispum* has revealed the presence of several classes of plant secondary metabolites such as flavonoids (quercetin, apiol, myristicin, apigenin, luteolin and their glycosides), coumarins, and terpenes [14]. These compounds have a wide spectrum of biological activities, including insecticidal, antiestrogenic, anticancer, antioxidant and antimicrobial activities [15,16,17].

The aim of this work was to assess the antioxidant properties, total phenolic and flavonoid contents of the methanolic extract and the different fractions of *P. crispum* grown in Egypt.

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials

*Petroselinum crispum* aerial parts were collected from Giza governorate, Egypt. A plant sample was identified by Mrs. Teraza Labib a botanist at Orman Garden Herbarium, Giza, Egypt. The voucher specimen was kept in Medicinal Chemistry Department, Theodor Bilharz Research Institute, Giza, Egypt. The plant parts were dried in shade, crushed with an electric mill to fine powder and kept in dry conditions for the extraction process.

### 2.2 Chemicals

DPPH (1, 1-diphenyl-2-picryl hydrazyl radical), Potassium persulphate and disodium hydrogen

phosphate were purchased from Bio Basic Inc. (Canada). 2, 2'- azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) and Folin-Ciocalteu reagent were purchased from Sigma -Aldrich (Steinheim, Germany). Ammonium molybdate, sodium carbonate, sodium nitrite, sodium hydroxide and aluminium chloride were purchased from Merck (Darmstadt, Germany). Rutin, gallic acid, and ascorbic acid were purchased from Sigma -Aldrich (St. Louis, USA).

### 2.3 Extraction and Fractionation Process

*Petroselinum crispum* (3 Kg) dry powder was extracted by 85% methanol (MeOH). The solvent was evaporated under vacuum till dry using rotatory evaporator (BUCHI, Switzerland), this step was repeated 6 times. The dried methanol extract (600 g) was defatted by petroleum ether. The defatted methanolic extract was fractionated using different organic solvents such as methylene dichloride (CH<sub>2</sub>Cl<sub>2</sub>), ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH). All fractions were evaporated till dry under reduced pressure, and kept in dry vials for estimation of their antioxidant activities, total phenolic and flavonoid contents.

### 2.4 Total Phenolic Content

The total phenolic content was estimated using Folin-Ciocalteu method according to Abdel-Gawad et al., with a little modification [18]. Briefly, 0.5 ml plant extract dissolved in methanol (500 µg/ml) was added to 2.5 mL of 10 fold diluted Folin-Ciocalteu reagent and 2 mL sodium carbonate (7.5%). The mixture was then incubated at 45°C for 45 min in dark with continuous shaking. The absorbance was measured at 765 nm against a standard solution of gallic acid and the results were the averages of triplicate analyses. The total phenolic content (TPC) of the different plant extracts was expressed as mg of gallic acid equivalent/g dry weight extract (mg GAE /g extract).

### 2.5 Total Flavonoid Content

The total flavonoid content was determined by using aluminum chloride colorimetric assay according to the method described by El-Sayed et al. [19]. The hydroxyl groups of flavonoids form a complex with aluminium chloride (AlCl<sub>3</sub>). A pink colour appeared upon the reaction with sodium nitrite. 500 µL of plant extract in methanol (500 µg/ml) was mixed with 150 µL NaNO<sub>2</sub> (5%) and 2 ml distilled water. After 6 min, 150 µL of

AlCl<sub>3</sub> (10%) was added. After 6 min, 2 ml of NaOH (4%) was finally added and the reaction mixture was diluted by 200 µL distilled H<sub>2</sub>O. The absorbance was measured at 510 nm after 15 min against a standard solution of rutin. The total flavonoid content (TFC) was expressed as mg rutin equivalent per gram extract (mg RE /g extract) and all experiments were carried out in triplicate.

### 2.6 DPPH Free Radical Scavenging Activity

1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) scavenging activity assay of the different extracts was measured according to the procedure described by El-Sayed et al. [19]. Various concentrations of each extract (2 mL) were added to 1 mL solution of 0.1 mmol L<sup>-1</sup> DPPH. An equal volume of methanol and DPPH served as a control. After 20 min of incubation at 37°C in the dark, the absorbance was recorded at 517 nm. The experiment was performed in triplicate. The DPPH radical scavenging activity was calculated and the SC<sub>50</sub> (concentration of sample required to scavenge 50 % of DPPH radicals) value was evaluated. Decrease of the DPPH solution absorbance indicates an increase of the DPPH radical scavenging activity.

### 2.7 ABTS Assay

The ability of various extracts to quench 2, 2'-azino-bis (3- ethylbenzthiazoline- 6-sulphonic acid) cationic radical (ABTS<sup>+</sup>) and using Trolox<sup>®</sup> as reference [20]. The ABTS<sup>+</sup> was generated by overnight interaction between ABTS (7 mM) and potassium persulphate (2.45 mM) then it was kept in dark at 5°C in refrigerator. The intense coloured ABTS stock solution was diluted by ethanol with ratio 1:70 and its absorbance was adjusted to 0.7±0.01 at 734 nm. Finally, 100 µL (500 µg/ ml) of each plant extract was mixed with 1 mL of ABTS solution in micro cuvette and the reduction in absorbance was measured exactly after 2.5 min against blank sample. Trolox<sup>®</sup> standard solution (final concentration 0-15 µM) in methanol was prepared and assayed at the same conditions. The absorbance of the resulting oxidised solutions was compared with Trolox<sup>®</sup> standard calibration curve. Results were expressed in terms of mmol Trolox<sup>®</sup> equivalent per 100 g dry weight of plant extract.

### 2.8 Total Antioxidant Capacity Assay

The total antioxidant capacity assay was evaluated by the phosphomolybdate method

according to Prieto et al. [21]. Each sample solution (0.5 mL) and ascorbic acid (500 mg mL<sup>-1</sup>) were combined with 5 mL of reagent (0.6 mol L<sup>-1</sup> sulfuric acid, 28 mmol L<sup>-1</sup> sodium phosphate and 4 mmol L<sup>-1</sup> ammonium molybdate). A typical blank solution contained 5 mL of reagent solution and an appropriate volume of the solvent used for the sample. All tubes were capped and incubated in a boiling water bath at 95°C for 90 min. After cooling the samples to room temperature, the absorbance of the solution of each sample was measured at 695 nm against the blank. The experiment was performed in triplicate. The antioxidant activity is expressed as equivalents of ascorbic acid (AAE).

## 2.9 Statistical Analysis

The statistical analyses were performed using IBM SPSS (25) software. The results were given as means ± standard deviation (SD) and all experimental analyses were carried out in triplicate.

## 3. RESULTS AND DISCUSSION

### 3.1 Total Phenolic Content

Phenolic compounds are the most abundant secondary metabolites in plants, playing an important role for growth and protection from the harmful effects of pathogens, parasites and UV-visible rays [22]. The human diet contains different group of phenolic compounds (flavonoids, phenolic acids, tannins, coumarins, curcuminoids, lignins and quinones) which have a wide distribution of health benefits and biological properties [23]. In the present study, the EtOAc fraction of *P. crispum* exhibited the

highest total phenolic content (121.95±2.15, mg GAE/ g extract) followed by BuOH fraction (92.06±1.17, mg GAE / g extract), CH<sub>2</sub>Cl<sub>2</sub> fraction (84.91±1.33, mg GAE / g extract), MeOH extract (46.63±0.2, mg GAE/ g extract), and residue had the lowest phenolic contents (44.56±1.36, mg GAE/ g extract) as shown in Fig. 1. Therefore, EtOAc fraction contains high polyphenolic compounds more than the other fractions.

### 3.2 Total Flavonoid Content

Flavonoids are a wide diverse class of natural compounds probably the most important natural polyphenols. All of these compounds exhibit a broad spectrum of chemical and biological properties including radical scavenging activity [24]. Total flavonoid content was determined using aluminium chloride method. Aluminium chloride will form stable complex with carbonyl group at C4 and hydroxyls at C3 (flavonols) and C5 in flavonols and flavones. It could also form labile acid complexes with hydroxyls in the ortho position in B rings of flavonoids [25]. The results in Fig. 1 showed that the total flavonoid content of EtOAc fraction yielded the highest total flavonoids content (106.45±2.18 mg RE / g extract). The yield of total flavonoids content was found to be in the order EtOAc fraction > BuOH fraction > CH<sub>2</sub>Cl<sub>2</sub> fraction > MeOH extract > residue.

### 3.3 DPPH Assay

The DPPH free radical scavenging assay is a powerful tool that has been typically used to evaluate the free radical scavenging activities of pure or mixed compounds. When antioxidants

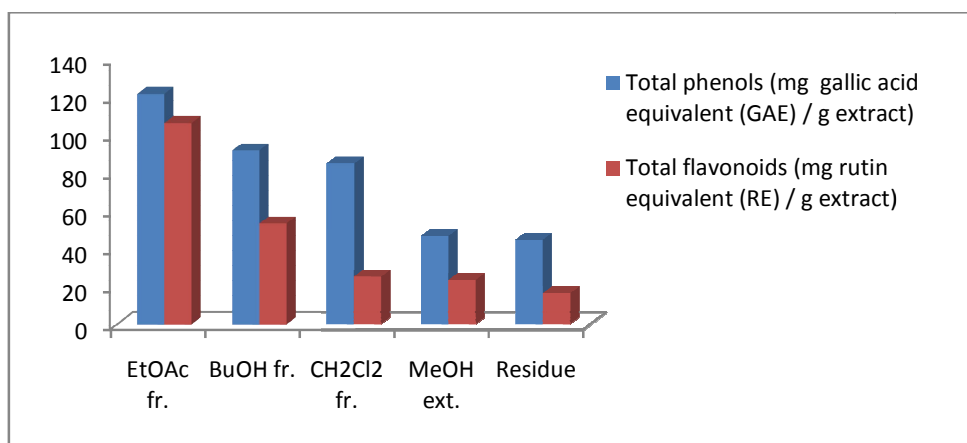
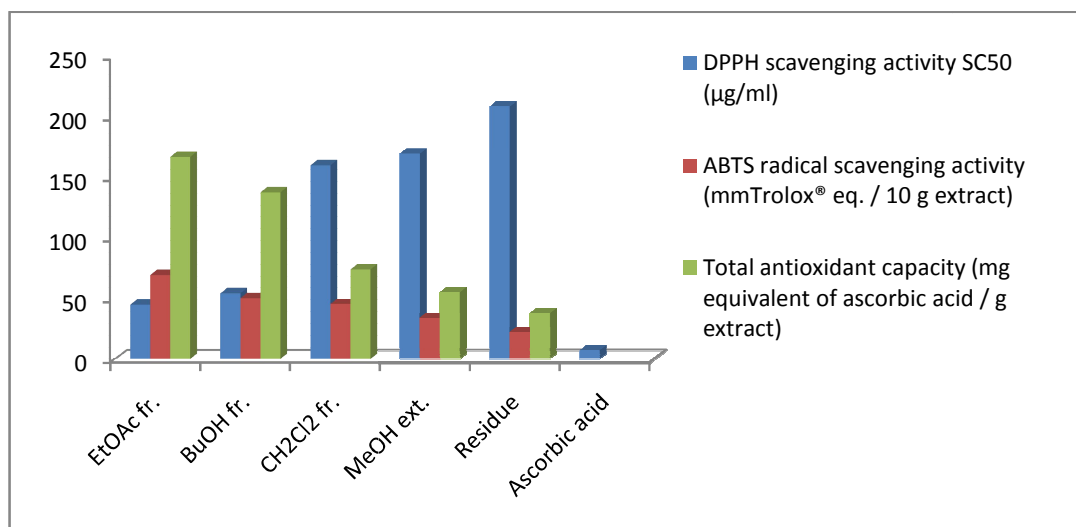


Fig. 1. Total phenolic and flavonoid contents of *P. crispum* extracts



**Fig. 2. DPPH scavenging activity, ABTS radical scavenging activity and total antioxidant capacity of *P. crispum* extracts**

react with DPPH (purple color), one or more hydrogen atoms are transferred from the antioxidants to DPPH, which results in the oxidation of antioxidants and the reduction of DPPH radicals to DPPH-H yellow colour [26]. *P. crispum* methanolic extract and its fraction showed moderate DPPH scavenging results (Fig. 2). The EtOAc fraction showed the highest antioxidant activity than other fractions ( $Sc_{50} = 44.51 \pm 2.06 \mu\text{g/mL}$ ), followed by BuOH fraction ( $Sc_{50} = 53.79 \pm 0.37 \mu\text{g/mL}$ ),  $\text{CH}_2\text{Cl}_2$  fraction ( $Sc_{50} = 159.72 \pm 3.74 \mu\text{g/mL}$ ), MeOH extract ( $Sc_{50} = 168.98 \pm 3.85 \mu\text{g/mL}$ ), and residue ( $Sc_{50} = 207.81 \pm 0.40 \mu\text{g/mL}$ ) compared to ascorbic acid ( $Sc_{50} = 6.79 \pm 0.17 \mu\text{g/mL}$ ). These results revealed that the ethyl acetate and butanolic fractions are the most active fractions.

### 3.4 ABTS Assay

The ABTS<sup>•+</sup> radical cation is greenish blue in colour and when it reacts with antioxidants it is able to transfer an electron to ABTS radical cation and the colour discharge according to the antioxidant concentration. The advantage of the ABTS radical is its high reactivity, and thus the ability to react with a broader range of antioxidants. On the other hand, the preparation of the ABTS reagent is more difficult and it is not stable compared to DPPH which lead to the unbiased results [27]. The results in Fig. 2 showed that the EtOAc and BuOH fractions has the highest antioxidant activity ( $69.82 \pm 3.07$  and  $50.06 \pm 2.47 \text{ mmTrolox}^{\text{®}}$  eq. / 10 g extract, respectively) whereas the residue showed the

lowest antioxidant activity ( $21.98 \pm 0.56 \text{ mmTrolox}^{\text{®}}$  eq. / 10 g extract). The results demonstrated that EtOAc fraction was a potent ABTS<sup>•+</sup> radical cation scavenger.

### 3.5 Total Antioxidant Capacity Assay

This assay is based on the reduction of phosphomolybdate ion in the presence of an antioxidant resulting in the formation of a green phosphate/MoV complex which is measured spectrophotometrically [28]. The results in Fig. 2 showed that the ethyl acetate fraction was the most active fraction ( $166.83 \pm 1.96 \text{ mg}$  of ascorbic acid eq. / g extract) and other fractions have the order of EtOAc fraction > BuOH fraction >  $\text{CH}_2\text{Cl}_2$  fraction > MeOH extract > residue. Therefore, these results suggested that phenolic compounds especially flavonoids may be the major contributors for the antioxidative potential and inhibitory actions towards the oxidative reactions.

## 4. CONCLUSION

The present study showed that the ethyl acetate and butanolic fractions of *P. crispum* have the highest total phenolic and flavonoid contents and antioxidant potential. The antioxidant properties of the methanolic extract and its derived fractions were well correlated with their total phenolic contents. Therefore, the daily consumption of *P. crispum* as spice in our food or supplements can help the antioxidant systems of the body to reduce the harmful effects oxidative stress.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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