



Research Article



## Antioxidant Activity, Total Phenolic and Flavonoid Contents of Three *Onobrychis* Species from Iran

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### ABSTRACT

**Background:** Plant phenolic compounds are a main group of plant natural products and flavonoids are the largest and best studied natural phenols. These substances possess a series of biological properties and act on biological systems as antioxidants. In present research, the aim is to determine in vitro total phenolics content and antioxidant activity of the methanolic extracts of three *Onobrychis* species belonging to the family Fabaceae, namely *O. sosnovskyi* Grossh., *O. viciifolia* Scop. and *O. melanotricha* Boiss. Furthermore, an attempt was made to identify any correlations between total phenolic content of the extracts with their antioxidant activities

**Methods:** Total phenol and flavonoid contents of the extracts were determined by Folin-Ciocalteu and aluminum chloride methods, respectively. Antioxidant activity was evaluated by three different test systems, namely 2, 2-diphenyl-1-picrylhydrazyl free radical scavenging (DPPH), metal-chelation activity and β-carotene/linoleic acid model.

**Results:** Results indicated that *O. viciifolia* extract contains the highest total phenolic content ( $10.38 \pm 0.33$  mg GAE/g of dry extract). However, the species are not remarkable different ( $P < 0.05$ ) in total flavonoid amount. The *Onobrychis* species represent strong antioxidant activity in DPPH radical scavenging ( $IC_{50}$ ;  $0.121 \pm 0.005$  to  $0.129 \pm 0.001$  mg/ml), metal chelating ( $IC_{50}$ ;  $1.10 \pm 0.006$  to  $1.80 \pm 0.003$  mg/ml) and also β-carotene bleaching methods. In addition, a high correlation was found between different antioxidant assays and total phenol content of the extracts ( $R^2 > 0.55$  to 0.98).

**Conclusion:** Our results showed that the examined *Onobrychis* extracts represent strong antioxidant activity; hence, they can be suggested as antioxidant agents for special use in future.

### Introduction

Free radicals such as reactive oxygen species (ROS) are formed naturally in the living cells that have important roles in cell signaling, also in environment some toxins may contain free radicals or stimulate the cells to produce free radicals.<sup>1</sup> High amount of these compounds are hazardous to the cells and damage all major its components, including nucleic acids, phospholipids, polypeptides chain and cell membranes that play a role in the development of diseases such as cancer and other health conditions.<sup>2</sup> Researches in the field of antioxidant advances focuses on antioxidant agents that can protect biological components from oxidative damage.<sup>3</sup> Antioxidants may act as scavenger, reducing, quencher agents and/or activators of cellular antioxidant enzymes to prevent from the free radical damages in biological systems.<sup>4,5</sup> Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) as synthetic antioxidants are used as additives in foods, but using of BHA and BHT are restricted because their toxic and carcinogenic effects.<sup>6</sup> Hence, there is a growing request and interest for found and

product of new antioxidants without harmful side effects in food and pharmaceutical applications.<sup>7</sup> Plants are main sources of compounds that act as antioxidant agents.<sup>8</sup> The important antioxidants that exist in plants are tocopherols and tocotrienols as fat-soluble antioxidants (vitamin E), folic acid (vitamin B<sub>9</sub>), L-ascorbic acid (vitamin C), flavonoids as the largest natural phenols, carotenoids, phenyl acrylic acids and phenyl methanoic acids.<sup>9</sup> The studies focused on the antioxidant effects of compounds derived from plants, because of their nutritional incidence and their role in health and disease.<sup>10-12</sup> Over the last few years, there has been an interest on antioxidant activity of the new compounds isolated from the Fabaceae family. The genus *Onobrychis* Miller (Fabaceae) includes about 170 annual or perennial mostly caulescent species constitutes a major group within the tribe Hedysareae DC. Members of the genus are mostly distributed in southwest Asia, Mediterranean region, and temperate Europe a few of which are cultivated as fodder or as ornamentals.<sup>13,14</sup> There are previous works on the chemical composition and antioxidant activity of *O.*

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*viciifolia*.<sup>15-17</sup> However, there is no reveal any references to previous works on the antioxidant activity of other *Onobrychis*. The aim of our study is to evaluate *in vitro* total phenolic content and antioxidant activity of the methanolic extracts from three *Onobrychis* species (*O. sosnovskyi*, *O. viciifolia* and *O. melanotricha*) and assess any correlations between total phenolic content in the extracts and their antioxidant activities.

## Materials and Methods

### Plant materials

Aerial parts of three *Onobrychis* species were collected from natural distribution areas. The data of the collected species are given in Table 1. The Voucher specimens of the species representing these collections have been deposited at the Bu Ali Sina University Herbarium (BASU), Hamedan, Iran.

### Preparation of extracts from *Onobrychis* species

Briefly, powdered aerial parts of plants (25 g) were extracted by absolute methanol (12 h), using a Soxhlet apparatus. Solvents of all extracts were removed by rotary evaporator (Lab Tech, Ev 311, Italy).

### Estimation of total phenol content

Determination of total phenol content was carried out by Folin- Ciocalteu reagent method.<sup>18</sup> In brief, 0.5 ml of each extract (1:10 g/ml) or Gallic acid was mixed with 5 ml Folin- Ciocalteu reagent (1:10 distilled water) and 4 ml of aqueous Na<sub>2</sub>CO<sub>3</sub> (1 M). The mixtures were left at room temperature for 15 min and the total phenol content was determined by colorimetric method at 765 nm (Perkin Elmer UV/visible spectrophotometer, USA). The calibration curve ( $y = 0.002x + 0.0177$ ,  $R^2 = 0.978$ ) was prepared using Gallic acid solutions at concentrations of 0 to 250 µg/ml in 50% methanol. Total phenol content was calculated from calibration curve and expressed as Gallic acid equivalent (mg GAE/g).

### Estimation of total flavonoids

The total flavonoid content was estimated using AlCl<sub>3</sub> method.<sup>19</sup> 0.5 ml of each extract (1:10 g/ml) in methanol were mixed with 1.5 ml of methanol, 0.1 ml of AlCl<sub>3</sub> (10%), 0.1 ml of KCH<sub>3</sub>COO (1 M) and 2.8 ml of distilled water and left at room temperature for 30 min. Absorbance of the mixtures was measured at 415 nm (Perkin Elmer UV/visible spectrophotometer, USA). The calibration curve ( $y = 0.0091x + 0.0206$ ,  $R^2 = 0.995$ ) was prepared using Quercetin solutions at concentrations of 12.5 to 100 µg/ml in methanol and

total flavonoid content was calculated from calibration curve and expressed as Quercetin equivalent (mg QE/g extract).

## Antiradical and Antioxidant activity

### Free radical scavenging capacity

Free radical scavenging activity of the extracts was measured according to Mensor *et al.*<sup>20</sup> In order to obtain dilutions, different extract concentrations were prepared in methanol (0.2 to 1 mg/ml) and 2.5 ml of each concentration was added to 1 ml of alcohol solution of DPPH (0.3 mM). After completion the samples were first kept in a dark place at room temperature for 30 min and bleaching of DPPH was read at 517 nm against a blank (methanol). The inhibition in percent for each concentration was calculated according to the following formula, DPPH free radical scavenging (%) =

$$\left[1 - \frac{(A_s - A_b)}{A_c}\right] \times 100 \quad \text{Eq.(1)}$$

Where, A<sub>s</sub> is absorbance of the reaction mixture containing 2.5 ml of extracts + 1 ml of DPPH, A<sub>b</sub> is absorbance of the reaction mixture containing 2.5 ml of extracts + 1 ml methanol and A<sub>c</sub> is absorbance of control sample containing 1 ml of DPPH + 2.5 ml methanol. Also IC<sub>50</sub> value, which represented the concentration of the sample that caused 50% inhibition, was determined. Tests were carried out in triplicate and ascorbic acid was used as a positive control.

### Iron chelating capacity

The chelation of ferrous ions by extracts in different concentration (0.2 to 1 mg/ml) was estimated by method of Dinis *et al.*<sup>21</sup>. Briefly, 0.05 ml of FeCl<sub>2</sub><sup>2+</sup> (2 mM) was added to 0.5 ml of different concentrations of the extracts. After 30 s, the reaction was initiated by the addition of 0.2 ml of ferrozine solution (5 mM). The mixture was left at room temperature and after 10 min; the absorbance at 562 nm was measured. The percentage inhibition of -Fe<sup>2+</sup>-ferrozine complex formation ability was calculated with the help of the following formula: Metal-chelating activity (%)

$$= \left[\frac{(A_c - A_s)}{A_c}\right] \times 100 \quad \text{Eq.(2)}$$

Where, A<sub>c</sub> was the absorbance of the control (Fe<sup>2+</sup>-Ferrozine complex without extract), and A<sub>s</sub> was the absorbance of Fe<sup>2+</sup>-Ferrozine complex mixed with extract/ascorbic acid.

### β-Carotene bleaching assay

Antioxidant activity of the samples was evaluated using the β-carotene/linoleic acid system.<sup>22,23</sup>

Table 1. The collections of the investigated *Onobrychis* species.

Species	Voucher number	Locality	Date	Altitude (m)
<i>O. sosnovskyi</i>	BASU14214	East Azerbaijan: 16 km to Marand	24.08.2013	1725
<i>O. viciifolia</i>	BASU19615	West Azerbaijan: Orumieh to Oshnavieh, 10 km after Sangar	23.08.2013	1650
<i>O. melanotricha</i>	BASU19263	Kordestan: Marivan to Saqqez, 60 km after Marivan	04.08.2013	1750

A mixture of  $\beta$ -carotene/linoleic acid was prepared by adding together of 0.5 mg  $\beta$ -carotene in 1 ml chloroform, 20 mg linoleic acid and 200 mg Tween 40. The chloroform was removed using a rotary evaporator apparatus under vacuum and 50 ml of oxygenated distilled water was subsequently added to the reaction mixture residue. 5 ml of the obtained emulsion added to 2 mg of extract/BHT dissolved in 0.4% (w/v) Tween 40 solution and mixed thoroughly. The test tubes were incubated in a water bath at 50 °C for 2 h together with a negative control (blank) contained 2 mg of an extract in 6 ml of Tween 40 solution. Tween 40 without extract was served as negative control. The absorbance values were measured at 470 nm (at 15 min intervals), starting immediately after sample preparation ( $t = 0$  min). Antioxidant activity (inhibition percentage, AA%) was calculated according to the following equation:

$$AA\% = 1 - \left[ \frac{(A_E^{t=0} - A_E^{t=t})}{(A_W^{t=0} - A_W^{t=t})} + \frac{(A_{BHT}^{t=0} - A_{BHT}^{t=t})}{(A_W^{t=0} - A_W^{t=t})} \right] \times 100 \quad \text{Eq.(3)}$$

Where,  $A_E$  is the absorbance of the extract,  $A_W$  is the absorbance of the water as negative control and  $A_{BHT}$  is the absorbance of BHT as positive control.

#### Statistical analysis

All data are the average of triplicate analyses. Statistical analysis of variance was performed using Student's t-test by SPSS program and  $p$  value < 0.05 was regarded as significant. Data are expressed as

means  $\pm$  standard deviation.

## Results

### Total phenol and flavonoid contents

Typical phenolic acids such as phenol and flavonoid compounds are known as antioxidant agents.<sup>24</sup> In this research, results indicated that the amount of total phenols varied among studied species and ranged from  $6.25 \pm 0.28$  to  $10.38 \pm 0.33$  mg GAE/g of dry extract. The highest total phenol content was found in *O. viciifolia* and the lowest in *O. melanotricha*. Also results showed that the three *Onobrychis* species are not remarkable different ( $P < 0.05$ ) in total flavonoids content. However, *O. viciifolia* possesses a higher amount of total flavonoids ( $5.08 \pm 0.64$  mg QES/g of dry extract) in comparison with two other species (Table 2).

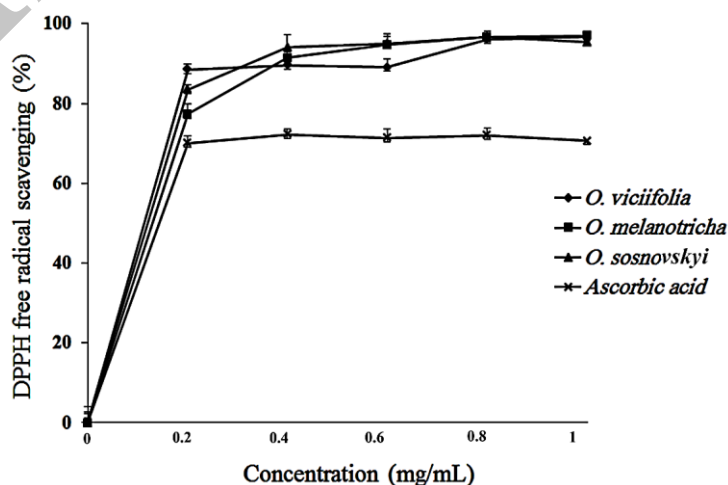
### Free radical scavenging capacity

In foods and biological systems, antioxidants especially free radical scavenging agents are very important due to their role in the deleterious of free radicals.<sup>25</sup> Results of antioxidant assay of the extracts showed the excellent concentration-dependent antiradical effect in comparison with ascorbic acid (71.26%) as a synthetic antioxidant (Table 2 and Figure 1). The greater antioxidant activity is reflected in a lower  $IC_{50}$ . All studied extracts demonstrated ability to scavenge DPPH free radicals, where most of them had  $IC_{50}$  value lower than the synthetic antioxidant Vitamin C.

**Table 2.** Total phenol and flavonoid contents and antioxidant activity of the *Onobrychis* species.

Species	Total phenol (mg/g dw)	Total flavonoid (mg/g dw)	$\beta$ -Carotene /linoleic acid	DPPH radical scavenging		Metal chelating activity	
				$IC_{50}$ (mg/m)	Percent (%)	$IC_{50}$ (mg/ml)	Percent (%)
<i>O. viciifolia</i>	$10.38 \pm 0.33^a$	$5.08 \pm 0.64^a$	$64.32 \pm 2.2^a$	$0.113 \pm 0.002^a$	92.70	$1.80 \pm 0.003^b$	19.13
<i>O. melanotricha</i>	$6.25 \pm 0.28^c$	$4.92 \pm 0.43^a$	$31.54 \pm 2.5^c$	$0.129 \pm 0.001^b$	92.27	$1.10 \pm 0.006^a$	14.62
<i>O. sosnovskyi</i>	$8.77 \pm 0.46^b$	$4.48 \pm 0.26^a$	$55.53 \pm 1.5^b$	$0.121 \pm 0.005^b$	91.38	$1.26 \pm 0.004^a$	22.34
Ascorbic acid	-	-	-	$0.143 \pm 0.007^c$	71.26	$2.29 \pm 0.009^b$	12.28

Experiment was performed in triplicate and expressed as mean  $\pm$  SD. Values in each column with different superscripts are significantly different ( $P < 0.05$ ).



**Figure 1.** DPPH radical scavenging ability of the *Onobrychis* extracts in different concentrations.

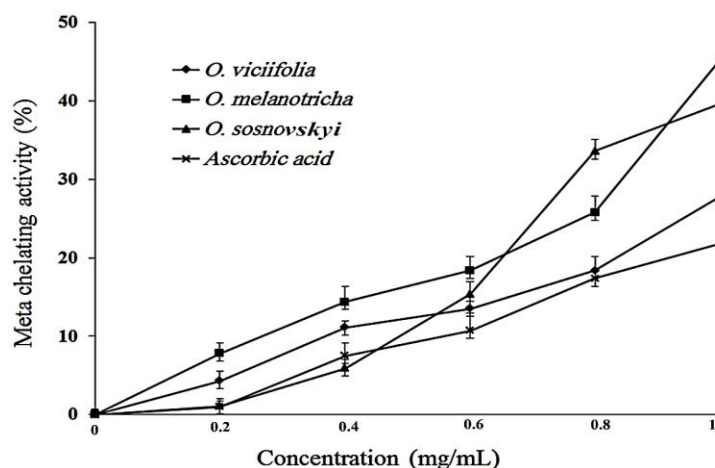


Figure 2. Metal chelating effects on ferrous ions of the *Onobrychis* extracts and ascorbic acid in different concentrations.

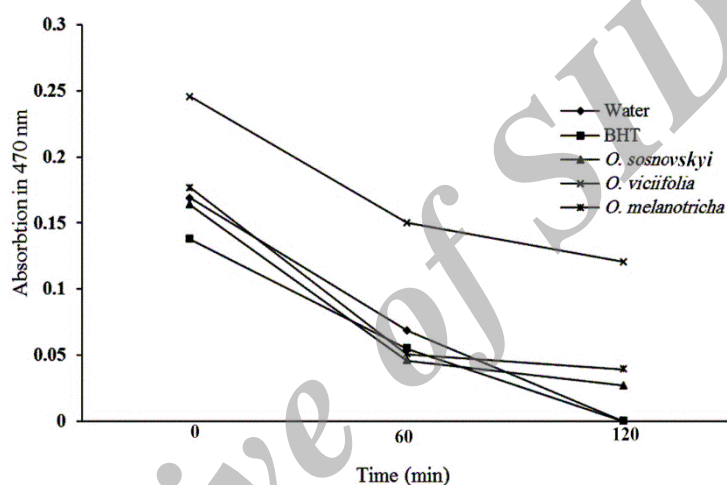


Figure 3. Inhibition of bleaching of  $\beta$ -carotene/linoleic acid emulsion by the *Onobrychis* extracts and BHT over 120 min.

The effectiveness of extracts as DPPH radical scavengers ranged in the following descending order: *O. viciifolia* ( $IC_{50}$ ;  $0.113 \pm 0.002$  mg/ml) > *O. sosnovskyi* ( $IC_{50}$ ;  $0.121 \pm 0.005$  mg/ml)  $\geq$  *O. melanotricha* ( $IC_{50}$ ;  $0.129 \pm 0.001$  mg/ml) > ascorbic acid ( $IC_{50}$ ;  $0.143 \pm 0.007$  mg/ml), (Table 2).

#### Iron chelating capacity

All investigated extracts were capable of chelating  $Fe^{2+}$  ions. Metal chelating property of the extracts was dependent on concentration (0.2 to 0.1 mg/ml) and linearly increased with the concentration increase (Table 2 and Figure 2). There was no significant difference ( $P < 0.05$ ) between metal chelating activity of *O. viciifolia* (14.62%,  $IC_{50}$ ;  $1.80 \pm 0.003$  mg/ml) and ascorbic acid (12.28%,  $IC_{50}$ ;  $2.29 \pm 0.009$  mg/ml) as a synthetic antioxidant; however the extracts from *O. melanotricha* (22.34%,  $IC_{50}$ ;  $1.10 \pm 0.006$  mg/ml) and *O. sosnovskyi* (19.13%,  $IC_{50}$ ;  $1.26 \pm 0.004$  mg/ml) showed significantly more chelating activity than that of *O. viciifolia* (Table 2).

#### $\beta$ -Carotene bleaching assay

Results from antioxidant activity by  $\beta$ -carotene/linoleic

acid system showed that *O. viciifolia* extract ( $64.32 \pm 2.2\%$ ) was more active than other two species (Table 2). Results from Amarowicz *et al.*<sup>23</sup> showed that the normalized antioxidant ability by  $\beta$ -carotene/linoleic acid method of selected plant extracts including some legumes at  $t = 60$  min and  $t = 120$  min of incubation are excellent. The change in absorbance ( $t = 0$  to  $t = 120$ ) of the reaction mixture in the presence of studied extracts, BHT (as positive control) and water (absence of the extracts) are shown in Figure 3. Here the methanolic extracts showed remarkable bleaching of  $\beta$ -carotene ( $P < 0.05$ ) in comparison with control (BHT as a synthetic antioxidant).

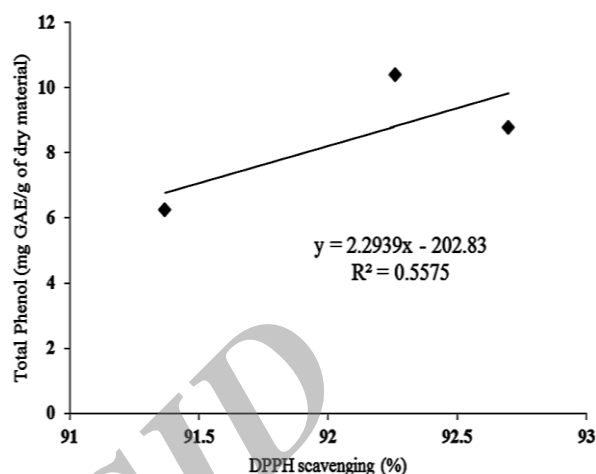
#### Discussion

Phenolic compounds in their chemical structure have hydroxyl groups that are power hydrogen donors and known as antioxidant agents. Antioxidants with hydrogen-donating ability can react with reactive oxygen and nitrogen species (ROS and RNS) and or chelate metals and inhibit lipid peroxidation.<sup>26,27</sup> These compounds are observed in different amounts and in various parts of plants. Phenolic structures can strongly interact with proteins and inhibit activity some

enzymes involved in ROS and RNS generation, such as various cytochrome P<sub>450</sub> isoforms, lipoxygenases, cyclooxygenase and xanthine oxidase.<sup>28</sup> Also, synergistic and anti-synergistic effects this compounds with other antioxidants and regulation of glutathione (as important antioxidant in animals, plants, fungi, and some bacteria) levels have been described.<sup>29,30</sup> Recent studies showed that differences in antioxidant potential of plant extracts could be related to the nature of the phenolic compounds such as flavonoids but not necessarily to their amounts.<sup>31</sup> Flavonoids are characterized by a phenyl benzopyran chemical structure with a pyran, pyrilium, or  $\gamma$ -pyrone center heterocycle.<sup>32</sup> Flavonoids have been classified into flavanols, flavanones, flavones, isoflavones, catechins, anthocyanins, proanthocyanidins. Pharmacological effects of flavonoids on human health are attributed to their potent antioxidant.<sup>33</sup> In this research, all extracts represented high total phenolic content. The highest content was detected in *O. viciifolia* ( $10.38 \pm 0.33$  mg GAE/g of dry extract) and the lowest in *O. melanotricha* ( $6.25 \pm 0.28$  mg GAE/g of dry extract). Our results indicated that the three species are not remarkable different ( $P < 0.05$ ) in total flavonoids amount (Table 2). In other studies on *O. viciifolia* extract by different solvents, the following chemical composition were found: the phenolic glycosides; kaempferol and quercetin, sucrose; the inositol [(+)-pinitol]; L-tryptophan, several flavonol glycosides and a mixture of condensed tannins.<sup>15-17,34,35</sup> Ince *et al.* assessed total phenolic acid content of different extracts (methanol, acetone, distillation water and ethyl ethanoate) of *O. viciifolia* using Folin-Ciocalteu method and reported its amount between  $11.35 \pm 0.82$  to  $38.26 \pm 0.15$   $\mu$ g Tannic acid/g of dry extract.<sup>17</sup> In the case of the *O. viciifolia*, the extractable compounds by acetone and methanol solvents had higher yield in comparison with ethyl ethanoate and distillation water solvents.<sup>17,36-38</sup>

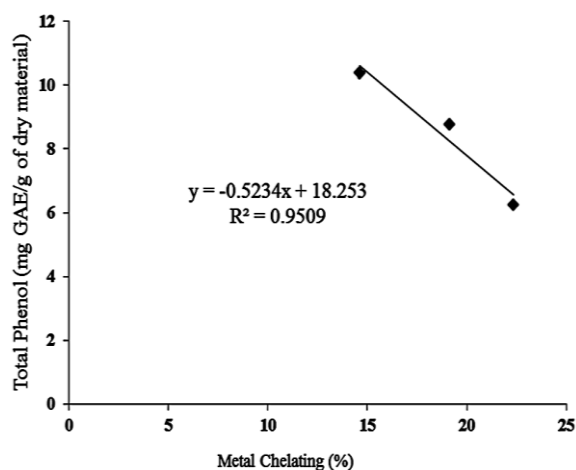
Antioxidant activity of *Onobrychis* extracts was assessed using DPPH radical scavenging method. *In vitro* antioxidant activity of the phenolic compounds on a molar basis is higher than vitamins E and C.<sup>39</sup> In this research, the extracts showed excellent concentration-dependent (0.2 to 1 mg/ml) antiradical properties and also the curve of antioxidant activity (in this evaluation system) of studied samples had overlap in some concentration. However, they were higher than vitamin C as a synthetic antioxidant (Figure 1). There is no study or reveal any references to previous works on the antioxidant activity (as DPPH free radical scavenging) of *O. melanotricha* and *O. sosnovskyi*. Whereas, a study on antioxidant activity of *O. viciifolia* reported its amount between 20 to 50% that showed excellent concentration-dependent (in 0.025, 0.05 and 0.1 mg/ml concentration) with antiradical property. This is in agreement with our results. Recent studies on the plant extracts have shown a positive relationship between the amount of phenolic content of the extracts and their

antioxidant properties, while there is poor or no correlation in other reports.<sup>40-42</sup> Here, there is a poor correlation coefficients ( $R^2 > 0.55$ ) between total phenolic content and DPPH free radical scavenging potential of the studied species (Figure 4).



**Figure 4.** Correlation between DPPH free radical scavenging and total phenolic content (mg GAE/g of dry extract) of the *Onobrychis* extracts.

Our results are in agreement with Babbar *et al.* who showed phenolic compounds alone are not fully responsible for antiradical activity of plant extracts. Other constituents such as reducing carbohydrates, tocopherols, ascorbates, carotenoids, terpenes, and pigments can be responsible for antioxidant activity. Nowadays, it is detected that the synergistic or antagonistic effect of any compounds in low amount should be considered for total antioxidant activities.<sup>43,44</sup> In physiological processes such as oxygen transport, cell respiration and activity of many enzymes, iron plays important roles. However, among the transition metals, iron is the most important factor in lipid peroxidation and oxidative damage to DNA, polypeptide chains and other cellular components.<sup>45</sup> Lipid oxidation and peroxides process is accelerated by produce hydroxide free radical by breaking down of hydrogen peroxide and ferrous state of iron, respectively ( $\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot\text{OH} + \cdot\text{OH}$ ).<sup>22</sup> Antioxidant agents such as some of phenolic acids compounds could inactive metal ions and their dependent processes.<sup>45</sup> In the metal chelating assay, ferrozine can quantitatively form complexes with  $\text{Fe}^{2+}$ . Plant extracts could inhibit complete formation of Ferrozine- $\text{Fe}^{2+}$  complex; this indicates their ability to chelate.<sup>46</sup> There is no report on the assessment of metal chelating properties of *Onobrychis* species studied here. Our results showed that the absorbance of Ferrozine- $\text{Fe}^{2+}$  complex decreased linearly and the extracts showed good metal chelating properties in a dose-dependent concentration (0.2 to 1 mg/ml), (Figure 2). The activity of studied methanolic extracts as metal chelators ranged in the following: *O. melanotricha* > *O. sosnovskyi*  $\geq$  *O. viciifolia* > ascorbic acid (Table 2).



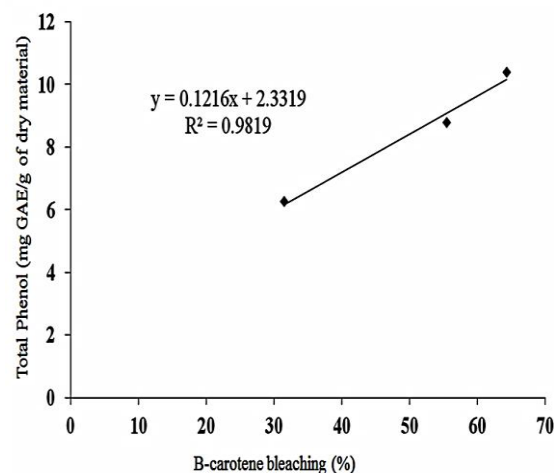
**Figure 5.** Correlation between metal chelating activity and total phenolic content (mg GAE/g of dry extract) of the *Onobrychis* extracts.

A high correlation ( $R^2 > 0.95$ ) existed between total phenolic content and metal chelating potential of the studied species (Figure 5).

Our results suggest that up to 90% of the metal chelating properties of the studied species are related to phenolic compounds. In other word it can be concluded the antioxidant activity of plant extracts is not limited to phenolics (10%). The antioxidant potential of the extracts (in 5 mg/ml concentration) was assessed by  $\beta$ -carotene/linoleic acid method. In this assay  $\beta$ -carotene/linoleic acid emulsion because of oxidation in the absence of an antioxidant generates free radicals and rapidly discolor.<sup>47</sup> Oxidation of linoleic acid was effectively inhibited by *O. viciifolia* extract ( $64.32 \pm 2.2\%$ ), followed by *O. sosnovskyi* ( $55.53 \pm 1.5\%$ ) and *O. melanotricha* ( $31.54 \pm 2.5\%$ ) extracts (Table 2). Ince *et al.* assessed antioxidant activity of different extracts of *O. viciifolia* using  $\beta$ -carotene bleaching assay and reported its amount between  $32.42 \pm 2.2\%$  to  $64.77 \pm 4.1\%$  in 0.05 mg/ml concentration of extracts ( $53.65 \pm 2.4\%$  for methanolic extract) and  $57.36 \pm 5.5$  to  $76.86 \pm 5.4$  in 0.1 mg/ml concentration of extracts ( $69.50 \pm 4.3$  for methanolic extract).<sup>17</sup> The high absorbance values or slow discoloration of  $\beta$ -carotene showed that the extracts have excellent antioxidant activity.

The change in absorbance (0 to 120 min) of  $\beta$ -carotene/linoleic acid emulsion in the presence and absence of studied extracts and BHT as positive control are shown in Figure 3.

There is a high correlation coefficients ( $R^2 > 0.98$ ) between total phenolic content of the extracts and  $\beta$ -carotene bleaching inhibition potential (Figure 6), suggesting that up to 98% of the inhibited bleaching of  $\beta$ -carotene in the species are related to phenolic compounds. To conclude, this study supports that the studied *Onobrychis* extracts presented high phenolic content and antioxidant properties. This is the first report that envisages the antioxidant activity of *O. sosnovskyi* and *O. melanotricha* and also *O. viciifolia* in metal chelating assay.



**Figure 6.** Correlation between  $\beta$ -carotene bleaching inhibition activity and total phenolic content (mg GAE/g of dry extract) of the *Onobrychis* extracts.

## Conclusion

Our work provides useful knowledge on phenolic content and antioxidant property of three *Onobrychis* species by easy, simple and reliable methods. Our observations implied that differences in the antioxidant activities between the extracts cannot adequately explain by quantitative difference in their total phenolic contents. Hence, the extracts of *Onobrychis* species include important phytochemicals that may help to develop new drug candidates for antioxidants, which might be helpful in treatment of diseases caused by different free radicals. Further studies are needed to evaluate the *in vivo* potential of these extracts in animal models.

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## Conflict of interests

The authors claim that there is no conflict of interest.

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