Antioxidant Properties and Principal Phenolic Phytochemicals of Iranian Tarragon (Artemisia dracunculus L.) Accessions

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Abstract. Artemisia dracunculus L. (tarragon), a common spice and vegetable grown throughout Iran, is an economically important species of the Asteraceae family. The free radical-scavenging activities of 12 Iranian \textit{A. dracunculus} accession extracts were evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP) assays. The total phenolic and flavonoid contents were measured using spectrophotometric techniques. Reversed-phase high-performance liquid chromatography (RP-HPLC) with UV detection was also used to identify the major phenolic compounds, revealing the presence of chlorogenic, syringic, and caffeic acids, while the predominant flavonoid was quercetin. Herniarin was the main coumarin in the extracts. Although a high antioxidant capacity was observed in all extracts, their antioxidant activities varied significantly, with the Birjand and Varamin accessions having the highest and the lowest capacities, respectively. The obtained total phenolic and flavonoid values similarly varied among tarragon accessions. Positive linear correlations were found between total antioxidant capacities (DPPH and FRAP assays) and total phenolic as well as flavonoid contents, indicating that phenolic compounds were the dominant antioxidant constituents in the tested plant extracts. Tarragon could therefore be a good source of natural antioxidants and has potential as a valuable dietary supplement.

Additional key words: coumarins, DPPH, flavonoids, FRAP, phenolic acids, radical scavenging activity

Introduction

Free radicals are highly reactive chemical species with an unpaired electron, which are ubiquitously generated by many redox processes in both biological and food systems (Mustafa et al., 2010). An over-production of free radicals can cause oxidative damage to biomolecules, including proteins, lipids, carbohydrates, and DNA, leading to chronic diseases, such as cancer, gastritis, cardiovascular diseases, neurodegenerative diseases, and inflammation, as well as premature aging (Silva et al., 2005; Jang et al., 2007; Stanojevic et al., 2009). In healthy individuals, several defense mechanisms are in place to protect against free radicals (Silva et al., 2005), while antioxidant-rich food and food additives can also play an important role in stabilizing free radicals, preventing them from damaging the body (Faudale et al., 2008; Karimi and Jaafar, 2011). Some synthetic antioxidant compounds such as butylated hydroxyanisole and butylated hydroxytoluene are commonly added to foodstuffs; however, in recent years, considerable interest has been directed toward investigating the use of natural antioxidants in preventive medicine and the food industry because of increasing safety concerns about the possible toxicities of synthetic antioxidants (Jang et al., 2007; Stanojevic et al., 2009; Gülcin et al., 2010).

Plants, including herbs and spices, contain many phytonutrients, which are potential sources of natural antioxidants (Erkan et al., 2008). Among them, the most widespread class of naturally occurring metabolites in plants are the phenols, with more than 8,000 phenolic structures currently known (Pereira et al., 2009; Dai and Mumper, 2010). Although phenolic compounds are classified as secondary metabolites, they are crucial for many important functional aspects of plant
life, including playing structural roles and being involved in defense strategies, as well as influencing the interaction between plants and their environment. Phenolic compounds contribute to the organoleptic and nutritive quality of food in terms of taste, aroma, bitterness, astringency, and color (Pandey and Rizvi, 2009; Dai and Mumper, 2010; Garcia-Salas et al., 2010). Phenolic compounds are broadly distributed throughout all plant organs and exhibit excellent antioxidant properties that are retained in plant-derived foods (Dai and Mumper, 2010). Extensive epidemiological studies have indicated that the intake of phenolic compounds is an important health-protecting factor that plays a major role in preventing human neurodegenerative diseases, coronary heart disease, and certain cancers (Velioglu et al., 1998; Aaby et al., 2004; Skerget et al., 2005; Katalinic et al., 2010). The structure of phenols is a key determinant of their antioxidant activity; phenolic compounds possess one or more aromatic rings with one or more hydroxyl groups, and their hydroxyl groups act as reducing agents, singlet oxygen quenchers, and hydrogen donors (Dai and Mumper, 2010; Garcia-Salas et al., 2010). The antioxidant activity of phenolic compounds is also attributable to their ability to inhibit enzymes and chelate metal ions involved in the formation of reactive oxygen species (Aaby et al., 2004; Pereira et al., 2009). Moreover, phenolic compounds are thought to have enhanced protective and regenerative effects when combined with other dietary antioxidants, namely ascorbic acid, β-carotene and α-tocopherol (Erkan et al., 2008; Pereira et al., 2009); therefore, plant extracts containing phenolics such as phenolic acids, flavonoids, and coumarins, as efficient free radical scavengers, are used as functional ingredients in various foods, medicines, beverages and cosmetics (Wu et al., 2007; Katalinic et al., 2010; Komes et al., 2011).

Tarragon (Artemisia dracunculus L.) has a long history of use as a spice and remedy. This perennial member of the Asteraceae (Obolskiy et al., 2011) is cultivated around the world, particularly in southern Europe, central Asia, Russia, and the USA, where its aromatic leaves are used in seasonings, salads, soup, sauces, and tarragon vinegar (Kordali et al., 2010; Chauhan et al., 2010; Obolskiy et al., 2011). A. dracunculus has a cool, spicy, licorice-like aroma, and is one of the 20 most commonly grown herbs in Europe (Gerard, 1987; Eisenman and Struwe, 2011; Obolskiy et al., 2011). A. dracunculus is safe to use as a dietary supplement, and possesses a wide range of health benefits as an herbal medicine, with uses as a sedative, carminative, digestive stimulant, antiepileptic, and an anticonvulsant, as well as possessing free radical-scavenging activities (Shahriyary and Yazdanparast, 2007; Aglarova et al., 2008; Chauhan et al., 2010; Obolskiy et al., 2011; full pharmacological and phytochemical characteristics and useful properties of A. dracunculus are reviewed by Aglarova et al., 2008 and Obolskiy et al., 2011). A. dracunculus extracts and individual compounds have been demonstrated to be potentially useful for decreasing the incidence of coronary and cardiovascular diseases in humans, blood platelet adhesion, plasma glucose levels, and lipid peroxidation (Eisenman and Struwe, 2011; Obolskiy et al., 2011; Weinoehrl et al., 2012).

Although the aerial parts of the plant contain a wide range of phytochemicals including monoterpenoids, sesquiterpenoids, phenolics, polyacetylenes, and alkaloids (Chauhan et al., 2010; Eisenman and Struwe, 2011), chemical analyses of A. dracunculus have revealed the main classes of its metabolic constituents to be coumarins, flavonoids, and phenolic acids. The aerial tissues of A. dracunculus usually contain >1% coumarins, including herniarin, coumarin, esculetin, and esculin. Moreover, flavonoids and phenolic acids, such as quercetin, luteolin, naringenin, camphorol, chlorogenic acid, caffeic acid, caffeoylquinic acid, and chioic acid, have also been reported in several studies (Onuchak et al., 2000; Aglarova et al., 2008; Obolskiy et al., 2011; Weinoehrl et al., 2012). Thus, A. dracunculus is considered to be an excellent source of phenolic compounds that could be used not only to preserve foods, but also to contribute to a healthy diet.

The identification and quantification of phenolic compounds in food and condiments are necessary to determine their effects on human health (Proestos et al., 2006). High-performance liquid chromatography (HPLC) is commonly used to analyze phenolics in plants (Spacil et al., 2008; Steinmann and Ganzera, 2011); however, a number of other methods have also been developed and applied to measure the antioxidant properties of plant extracts (Cao et al., 1993; Miller et al., 1993; Brand-Williams et al., 1995; Ghiiselli et al., 1995; Benzie and Strain, 1996). Since plant-derived antioxidants exert their effects through a variety of different mechanisms, assaying their antioxidant activities using more than one method is important (Frankel and Meyer, 2000; Trumbeckaite et al., 2011).

In this study, Iranian A. dracunculus accessions were screened as potential sources of natural antioxidants, in terms of both their total contents of phenolic compounds and flavonoids, as well as their antioxidant activity. Furthermore, qualitative and quantitative analyses of major phenolic acids, flavonoids, and coumarins from the aerial part of the plant were performed using HPLC. Finally, we assayed the relationship between antioxidant activity and the phenolic compounds of these tarragon accessions.

**Materials and Methods**

**Plant materials**

Rhizomes of 12 Iranian accessions of A. dracunculus L. (Table 1), previously selected for their medicinal properties, were supplied by the Medicinal Plants and Drug Research Institute of Shahid Beheshti University, Tehran, Iran, and...
were cultivated in field conditions at the horticultural sciences research center of the University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran, in 2014. The aerial parts, consisting of the leaves and upper portions of shoots and inflorescences, were collected when the plants were in full bloom, then air-dried in the shade, stored in paper bags, and stored in a dark room at room temperature.

Reagents, Solvents and Standards

Folin-Ciocalteu reagent, saturated sodium carbonate, aluminum chloride, potassium acetate, ferric chloride, acetate buffer, and iron sulfate were supplied by Thermo Fisher Scientific (MA, USA). Analytical grade 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-tripryridyl-s-triazine (TPTZ), as well as hydrochloric acid, rutin, gallic acid, caffeic acid, chlorogenic acid, ascorbic acid, syringic acid, vanillic acid, ferulic acid, chicoric acid, p-coumaric acid, quercetin, luteolin, myricetin, apigenin, naringenin, herniarin, and coumarin were purchased from Sigma-Aldrich (MO, USA). All other reagents and organic solvents were of analytical grade and were obtained from Thermo Fisher Scientific (MA, USA). Water was prepared using a Millipore plus system (Merck Millipore, MA, USA).

Extraction Process

Aqueous methanol was used as an extraction solvent, and was previously shown to efficiently extract phenolic compounds from a range of plant materials (Garcia-Salas et al., 2010; Khoddami et al., 2013). For each accession, 10 g dried plant material was ground to a fine powder and dissolved in 100 mL of 80% methanol. The extraction was carried out by shaking the sample at room temperature for 72 h. After filtration through filter paper (GF/A, 110 mm; Whatman, Maidstone, UK), the clear extract was concentrated in a vacuum rotary evaporator at 50°C. The remaining extract was then dried in a vacuum oven at 40°C for 3 h to ensure the removal of any residual solvent. The resulting residues were used in the antioxidant activity assay and HPLC analysis. Three extraction replicates were prepared for each accession.

DPPH Radical Scavenging Assay

The procedure of Choi et al. (2002) was adapted for evaluating the free radical-scavenging capacity of the extracts. Briefly, various concentrations of each extract (ranging from 0.01-1.0 mg mL⁻¹) were prepared in methanol, then 2.5 mL of the extract (or methanol for the control) and 1.0 mL of a 3.0×10⁻³ M DPPH solution in methanol were vigorously mixed and left for 30 min in the dark at 25°C. The absorbance of each sample (As) was recorded at 517 nm using a UV-vis spectrophotometer (UV-1800; Shimadzu Corporation, Kyoto, Japan). The absorbance of a blank solution containing 1.0 mL methanol plus 2.5 mL plant extract solution was also recorded (Ab), while the DPPH solution plus methanol was used as the control (Ac). Ascorbic acid was used as a positive control, and three replicates were recorded for each sample. The radical scavenging capacity (RSC) of each plant extract was calculated using the following equation:

\[
\text{RSC} = \frac{\text{As} - \text{Ab}}{\text{Ac} - \text{Ab}} \times 100
\]

Table 1. Radical scavenging activities and total phenolic and flavonoid contents of extracts from 12 Iranian A. dracunculus accessions

<table>
<thead>
<tr>
<th>Accession No.</th>
<th>Origin (City)</th>
<th>DPPH assay x</th>
<th>FRAP assay y</th>
<th>Total phenolic content z</th>
<th>Total flavonoid content x</th>
<th>TFIC/TPhC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Esfahan</td>
<td>0.069±0.014</td>
<td>109.5±7.7</td>
<td>68.5±3.5</td>
<td>39.05±2.9</td>
<td>0.56</td>
</tr>
<tr>
<td>2</td>
<td>Abadeh</td>
<td>0.097±0.009</td>
<td>79.83±6.4</td>
<td>49.46±2.8</td>
<td>32.48±2.7</td>
<td>0.65</td>
</tr>
<tr>
<td>3</td>
<td>Samirom</td>
<td>0.069±0.02</td>
<td>101.97±6.4</td>
<td>57.74±2.2</td>
<td>36.79±2.7</td>
<td>0.63</td>
</tr>
<tr>
<td>4</td>
<td>Neyshaboor</td>
<td>0.065±0.017</td>
<td>113.12±5.6</td>
<td>60.05±3.3</td>
<td>38.57±2.7</td>
<td>0.64</td>
</tr>
<tr>
<td>5</td>
<td>Zarand</td>
<td>0.063±0.017</td>
<td>125.01±5.9</td>
<td>78.59±1.6</td>
<td>45.29±3.1</td>
<td>0.57</td>
</tr>
<tr>
<td>6</td>
<td>Estahbanat</td>
<td>0.077±0.009</td>
<td>85.19±5.1</td>
<td>51.94±2.7</td>
<td>35.16±1.8</td>
<td>0.67</td>
</tr>
<tr>
<td>7</td>
<td>Unknown</td>
<td>0.073±0.008</td>
<td>87±7.5</td>
<td>55.15±3.1</td>
<td>34.37±2.1</td>
<td>0.62</td>
</tr>
<tr>
<td>8</td>
<td>Birjand</td>
<td>0.039±0.007</td>
<td>148.29±6.6</td>
<td>96.52±4.7</td>
<td>50.4±1.6</td>
<td>0.52</td>
</tr>
<tr>
<td>9</td>
<td>Kermanshah</td>
<td>0.085±0.006</td>
<td>70.43±6.2</td>
<td>43.51±3.6</td>
<td>26.98±2.5</td>
<td>0.62</td>
</tr>
<tr>
<td>10</td>
<td>Varamin</td>
<td>0.101±0.010</td>
<td>68.04±4.5</td>
<td>40.91±3.1</td>
<td>25.44±1.9</td>
<td>0.62</td>
</tr>
<tr>
<td>11</td>
<td>Yazd</td>
<td>0.069±0.007</td>
<td>81.16±3.4</td>
<td>47.41±3.2</td>
<td>27.32±1.8</td>
<td>0.57</td>
</tr>
<tr>
<td>12</td>
<td>Hamadan</td>
<td>0.054±0.009</td>
<td>121±4</td>
<td>65.77±3.2</td>
<td>41.59±2.6</td>
<td>63</td>
</tr>
</tbody>
</table>

Ascorbic acid 0.034±0.005 397.5±13

x Data expressed as mean ± standard deviation (SD) of three replicates.
y Radical scavenging activity assayed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method (data expressed as IC50 mg mL⁻¹).
z Radical scavenging activity assayed by ferric reducing antioxidant power (FRAP) method (data expressed as μmol Fe²⁺ g⁻¹ dry weight).

**Data expressed as mg of gallic acid equivalents g⁻¹ dry weight.

**Data expressed as mg of rutin equivalents g⁻¹ dry weight.
acetate trihydrate (C\textsubscript{4}mM acetate buffer was prepared by mixing 3.1 g of sodium/g41/g79/g68/g89/g82/g81/g82/g76/g71/g3 /g38/g82/g81/g87/g72/g81/g87/g51/g75/g92/g87/g82/g70/g75/g72/g80/g76/g70/g68/g79/g3 /g54/g70/g85/g72/g72/g81/g76/g81/g74/g3 /g36/g81/g87/g76/g82/g91/g76/g71/g68/g81/g87/g3 /g51/g82/g90/g72/g85/g3 /g11/g41/g53/g36/g51/g12/g3 /g36/g86/g86/g68/g92

The total flavonoid content of the extracts was determined spectrophotometrically according to the aluminum chloride colorimetric method (Quettier-Deleu et al., 2000), with slight modification. A 1 mL aliquot of each plant extract was mixed with 2 mL 2% aluminum chloride hexahydrate methanolic solution and 6 mL 5% potassium acetate. After a 40 min incubation at room temperature, the absorbance of the reaction mixture was measured at 415 nm with a UV-vis spectrophotometer (UV-1800). The flavonoid content of the extracts was calculated using a rutin calibration curve. The results were expressed in mg of rutin equivalents (RE) g\textsuperscript{-1} dry extract. Samples were analyzed in three replications.

**Ferric-reducing Antioxidant Power (FRAP) Assay**

The ability of the extracts to reduce ferric ions was measured following the methodology of Benzie and Strain (1996), with slight modification. Fresh FRAP reagent was produced by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ, and 20 mM FeCl\textsubscript{3}-6H\textsubscript{2}O in a ratio of 10:1:1 at 37°C. The 300 mM acetate buffer was prepared by mixing 3.1 g of sodium acetate trihydrate (C\textsubscript{4}H\textsubscript{2}NaO\textsubscript{3}-3H\textsubscript{2}O) with 16 mL glacial acetic acid and was brought to a final volume of 1 L using double-distilled water. The TPTZ solution was prepared by making a solution of 10 mM TPTZ in 40 mM HCl. A total of 190 µL FRAP reagent was added to each well of a 96-well microtitre plate. To each well, 10 µL of plant extract was then added, incubated for 8 min at room temperature, and the absorbance was read at a wavelength of 593 nm using Bio-Rad (Hercules, CA, USA) microtitre plate reader. A blank reading was taken, and aqueous solutions of ferrous sulphate heptahydrate (25-1000 µM) were used to generate a calibration curve. The antioxidant power of the extract was expressed as µM Fe (II). All determinations were performed in triplicate.

**Phytochemical Screening**

**Total phenolic content:** The Folin-Ciocalteu method (Spanos and Wrolstad, 1990) was used to determine the phenolic content of the A. dracunculus extracts. A 10 µL aliquot of each sample extract was mixed with 490 µL distilled water and 500 µL Folin-Ciocalteu reagent. After adding 500 µL saturated sodium carbonate (1%), the resulting mixture was vortexed for 1 min and then incubated for 2 h at room temperature. The absorbance of the samples was measured at 765 nm using a UV-vis spectrophotometer (UV-1800). The total phenolic results were expressed as gallic acid equivalents (mg GAE·g\textsuperscript{-1} plant dry weight [DW]), according to the gallic acid standard curve. The procedure was performed in triplicate.

**Flavonoid Content**

The total flavonoid content of the extracts was determined spectrophotometrically according to the aluminum chloride colorimetric method (Quettier-Deleu et al., 2000), with slight modification. A 1 mL aliquot of each plant extract was mixed with 2 mL 2% aluminum chloride hexahydrate methanolic solution and 6 mL 5% potassium acetate. After a 40 min incubation at room temperature, the absorbance of the reaction mixture was measured at 415 nm with a UV-vis spectrophotometer (UV-1800). The flavonoid content of the extracts was calculated using a rutin calibration curve. The results were expressed in mg of rutin equivalents (RE) g\textsuperscript{-1} dry extract. Samples were analyzed in three replications.

**RP-HPLC Analysis of Phenolic Compounds**

The dried extracts were dissolved in methanol and analyzed using a PerkinElmer series 200 HPLC (PerkinElmer, Inc., MA USA). The HPLC system consisted of a Quaternary LC Pump Model 200Q/410, a SIL-20A XR auto-sampler, a SPD-M20A VP photodiode array detector, and a CL-20A VP controller connected to a PC. A reverse phase Gemini 5U C\textsubscript{6}-Phenyl column (250×4.60 mm, 5 µm, 110A) was used. The data were monitored using TotalChrom software. A 10 µL volume of the filtered samples was injected, and the chromatogram was acquired at 280 nm and 350 nm. The mobile phase was composed of H\textsubscript{2}O (solvent A) and 20 mM phosphoric acid (solvent B). The program started with an isocratic elution using 90% B (0-3 min), which was linearly decreased to 5% over 50 min, then kept constant for 5 min. The elution was ramped back up to 90% B during 59-69 min. The elution was carried out at 25°C with a flow rate of 0.1 mL·min\textsuperscript{-1}. After each injection, the column was equilibrated for 10 min. The retention times and peak areas of pure phenolic standards were compared with those of each extract to identify and quantify the compounds in the sample. All samples and standards were injected in triplicate. The results were expressed in term of mg compound·100 g\textsuperscript{-1} DW.

**Statistical Analysis**

All data are presented as the mean ± standard deviation of three replicates. Correlation analyses of radical scavenging activities and total phenolic and flavonoid contents were carried out using Microsoft Office Excel 2013.

**Results**

**Antioxidant Activity**

**DPPH radical scavenging:** The free radical-scavenging activities of the 12 tarragon accession extracts were determined using a DPPH assay (Table 1). The IC\textsubscript{50} (50% reduction in DPPH concentration) values indicated free radical-scavenging activity in all the plant extracts but at different levels. The highest scavenging activity was observed for ascorbic acid itself (with an IC\textsubscript{50} at 0.034 ± 0.005 mg·mL\textsuperscript{-1}), while the Birjand accession showed the highest antioxidant activity of the plant extracts (IC\textsubscript{50} at 0.039 ± 0.007 mg·mL\textsuperscript{-1}), followed by the Hamadan accession (IC\textsubscript{50} at 0.054 ± 0.009 mg·mL\textsuperscript{-1}). The lowest antioxidant activity was observed in the Varamin accession (IC\textsubscript{50} at 0.101 ± 0.010 mg·mL\textsuperscript{-1}).

\[ RSC = 100 \left(1 - \frac{As - Ab}{Ac}\right) \]
Table 2. Quantitative analysis of major phenolic compounds identified in extracts from 12 Iranian A. dracunculus accessions

<table>
<thead>
<tr>
<th>Accession</th>
<th>Chlorogenic acid</th>
<th>Syringic acid</th>
<th>Caffeic acid</th>
<th>Vanillic acid</th>
<th>Ferulic acid</th>
<th>p-Coumaric acid</th>
<th>Herniarin</th>
<th>Coumarin</th>
<th>Naringenin</th>
<th>Luteolin</th>
<th>Quercetin</th>
<th>Apigenin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esfahan</td>
<td>15.94±0.58</td>
<td>11.48±0.08</td>
<td>n.d.</td>
<td>12.91±0.09</td>
<td>9.46±0.05</td>
<td>18.18±0.07</td>
<td>n.d.</td>
<td>69.52±1.39</td>
<td>n.d.</td>
<td>107.82±1.03</td>
<td>5.1±0.05</td>
<td>n.d.</td>
</tr>
<tr>
<td>Abadeh</td>
<td>26.03±0.07</td>
<td>9.8±0.31</td>
<td>n.d.</td>
<td>18.55±0.07</td>
<td>1.64±0.01</td>
<td>tr.</td>
<td>n.d.</td>
<td>58.88±0.84</td>
<td>15.39±0.05</td>
<td>81.62±0.07</td>
<td>5.98±0.04</td>
<td>n.d.</td>
</tr>
<tr>
<td>Samirom</td>
<td>30.15±1.01</td>
<td>3.17±0.07</td>
<td>n.d.</td>
<td>15.94±0.16</td>
<td>22.89±0.4</td>
<td>0.24±0.01</td>
<td>n.d.</td>
<td>68.09±1.91</td>
<td>12.7±0.08</td>
<td>58.87±1.09</td>
<td>0.11±0.01</td>
<td>tr.</td>
</tr>
<tr>
<td>Neyshaboer</td>
<td>24.27±0.09</td>
<td>9.58±0.49</td>
<td>n.d.</td>
<td>13.42±0.08</td>
<td>4.57±0.04</td>
<td>0.46±0.02</td>
<td>n.d.</td>
<td>36.68±0.63</td>
<td>39.46±0.74</td>
<td>4.37±0.06</td>
<td>223.78±1.73</td>
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</tr>
<tr>
<td>Zarand</td>
<td>27.58±0.36</td>
<td>3.36±0.11</td>
<td>1.33±0.04</td>
<td>13.27±0.07</td>
<td>18.83±0.08</td>
<td>1.42±0.03</td>
<td>3.53±0.05</td>
<td>39.77±0.78</td>
<td>3.27±0.05</td>
<td>59.05±0.08</td>
<td>13.4±0.07</td>
<td>0.1±0.01</td>
</tr>
<tr>
<td>Eshtehabat</td>
<td>37.07±0.28</td>
<td>4.12±0.09</td>
<td>n.d.</td>
<td>15.47±0.14</td>
<td>26.44±1.02</td>
<td>1.07±0.06</td>
<td>18.19±0.33</td>
<td>61.41±1.36</td>
<td>0.93±0.02</td>
<td>75.72±1.42</td>
<td>tr.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Unknown</td>
<td>18.05±0.41</td>
<td>7.9±0.61</td>
<td>n.d.</td>
<td>9.57±0.43</td>
<td>tr.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>36.2±0.08</td>
<td>n.d.</td>
<td>92.55±0.63</td>
<td>tr.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Birjand</td>
<td>13.15±0.06</td>
<td>7.84±0.06</td>
<td>3.13±0.11</td>
<td>11.56±0.22</td>
<td>tr.</td>
<td>n.d.</td>
<td>34.85±0.6</td>
<td>2.04±0.01</td>
<td>n.d.</td>
<td>53.98±0.51</td>
<td>10.5±0.09</td>
<td>n.d.</td>
</tr>
<tr>
<td>Kermanshah</td>
<td>13.13±0.08</td>
<td>13.55±0.08</td>
<td>n.d.</td>
<td>21.28±0.03</td>
<td>tr.</td>
<td>n.d.</td>
<td>32.12±0.82</td>
<td>93.55±1.94</td>
<td>37.22±1.04</td>
<td>562.59±1.78</td>
<td>8.94±0.26</td>
<td>n.d.</td>
</tr>
<tr>
<td>Varamin</td>
<td>5.73±0.03</td>
<td>29.01±0.26</td>
<td>n.d.</td>
<td>3.16±0.06</td>
<td>56.73±1.12</td>
<td>20.11±0.34</td>
<td>93.74±1.05</td>
<td>0.48±0.01</td>
<td>n.d.</td>
<td>416.38±2.88</td>
<td>12.43±0.02</td>
<td>n.d.</td>
</tr>
<tr>
<td>Yazd</td>
<td>15.63±1.09</td>
<td>10.47±0.18</td>
<td>n.d.</td>
<td>5.66±0.09</td>
<td>0.14±0.01</td>
<td>7.56±0.22</td>
<td>33.08±0.3</td>
<td>3.32±0.06</td>
<td>6.35±0.02</td>
<td>201.58±3.03</td>
<td>5.33±0.08</td>
<td>n.d.</td>
</tr>
<tr>
<td>Hamadan</td>
<td>33.6±0.42</td>
<td>3.54±0.05</td>
<td>n.d.</td>
<td>25.05±0.39</td>
<td>19.0±1.47</td>
<td>4.7±0.03</td>
<td>84.75±1.13</td>
<td>7.59±0.01</td>
<td>130.59±0.07</td>
<td>6.08±0.71</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

All data expressed as mean ± standard deviation (SD) of three replicates in mg·100 g⁻¹ dry plant material. n.d. Not detected. tr. Trace.

FRAP Assay

The ferric-reducing properties varied considerably between the A. dracunculus accessions. The highest FRAP value (148.3 ± 6.6 µmol Fe²⁺·g⁻¹ DW) was observed in the Birjand accession followed by the Zarand accession (125.0 ± 5.9 µmol Fe²⁺·g⁻¹ DW). Among the remaining accessions, Hamadan also had a remarkably higher FRAP value (121 ± 4 µmol Fe²⁺·g⁻¹ DW) than the other tarragon accessions. In contrast, the lowest ferric reducing activity was observed in Varamin (68.0 ± 4.5 µmol Fe²⁺·g⁻¹ DW).

Total Phenolic and Flavonoid Contents

The total phenolic contents, calculated using the standard curve of GAE content, varied widely in the tarragon accessions, ranging from 40.9 to 96.5 mg GAE·g⁻¹ DW (Table 1). The highest phenolic content was detected in the Birjand accession (96.5 ± 4.7 mg GAE·g⁻¹ DW), followed by Zarand (78.6 ± 1.6 mg GAE·g⁻¹ DW) and Esfahan (68.5 ± 3.5 mg GAE·g⁻¹ DW). The lowest phenolic content was found in the Varamin extract (40.9 ± 3.1 mg GAE·g⁻¹ DW). The highest content of flavonoids was determined in Birjand (50.4 ± 1.6 mg RE·g⁻¹ DW), while Varamin had the lowest flavonoid content (25.4 ± 1.9 mg RE·g⁻¹ DW). The ratio of total flavonoid content to total phenolic content (TFIC/TPhC) ranged from 52% to 67% of the total phenolic content of different A. dracunculus accession extracts.

Correlations

Statistical analysis revealed significant relationships between the antioxidant activities and total phenolic and flavonoid contents of the tarragon methanolic extracts (r²=0.7059 for DPPH vs TPhC; r²=0.7548 for DPPH vs TFIC; r²=0.7965 for FRAP vs TFIC; r²=0.8866 for FRAP vs TFIC; r²=0.827 for DPPH vs FRAP; r²=0.835 for TFIC v TPhC; Fig. 1).

HPLC Analyses of the Major Phenolic Compounds

HPLC analysis was performed to get an insight into the phenolic profiles of the A. dracunculus accessions. Seven phenolic acids (chlorogenic acid, syringic acid, choric acid, caffeic acid, vanillic acid, ferulic acid, p-coumaric acid), and four flavonoids (luteolin, quercetin, naringenin, apigenin), as well as coumarin and herniarin were identified and quantified (Table 2). A considerable variation was observed in the phenolic compounds of the tarragon accessions. Chlorogenic acid (ranging from 5.73 ± 0.03 to 37.07 ± 0.28 mg·100 g⁻¹ DW), syringic acid (3.17 ± 0.07 to 29.01 ± 0.26 mg·100 g⁻¹ DW), and caffeic acid (3.16 ± 0.06 to 25.05 ± 0.39 mg·100 g⁻¹ DW) were the dominant phenolic acids detected in all accessions. Ferulic acid and vanillic acid were found to represent up to 56.73 ± 1.12 mg·100 g⁻¹ and 26.44 ± 1.02 mg·100 g⁻¹ DW, respectively, but were not present in all accessions. Luteolin (58.87 ± 0.09 to 562.59 ± 1.78 mg·100 g⁻¹ DW) was the predominant flavonoid among the accessions, and all lines except for Esfahan and the accession of unknown origin (accession 7) contained coumarin (0.48 ± 0.01 to 39.46 ± 0.74 mg·100 g⁻¹ DW). The contents of herniarin, a major compound of tarragon extracts, ranged from 33.08 ± 0.03 to 93.74 ± 1.05 mg·100 g⁻¹ DW.
Fig. 1. Correlation between radical scavenging activities and phytochemical components of different 12 Iranian A. dracunculus accessions carried out using Microsoft Office Excel 2013. (A) Correlation between flavonoid and antioxidant activity measured by the inhibition of 1,1-diphenyl-2-picrylhydrazyl (DPPH). (B) Correlation between flavonoid content and antioxidant activity measured by ferric reducing antioxidant power (FRAP). (C) Correlation between total phenolic content and antioxidant activity (DPPH). (D) Correlation between total phenolic content and antioxidant activity (FRAP). (E) Correlation between antioxidant tests DPPH and FRAP. (F) Correlation between total phenolic and flavonoid contents. DW, dry weight; GAE, gallic acid equivalent; RE, rutin equivalent.

Discussion

Oxidative damage by free radicals is considered to cause premature aging (Finkel and Holbrook, 2000; Wu et al., 2006) and numerous serious diseases, including cancer and cardiovascular disease (Parejo et al., 2002; Wu et al., 2007). The ability of natural antioxidants to scavenge and quench free radicals may prevent these health issues (Gülçin et al., 2010); therefore, we utilized two radical scavenging methods, DPPH scavenging and FRAP scavenging, to evaluate the antioxidant activity of tarragon accession extracts in this study.

The relatively stable free radical compound, DPPH, has been widely used to determine the free radical-scavenging ability of various plant extracts (Hatano et al., 1988; Amarowicz et al., 2004; Jang et al., 2007; Ghahremani-majd et al., 2012). The ability of the tarragon extracts to scavenge DPPH was assessed on the basis of their IC50 values, defined as the concentration of plant extract needed to decrease the initial DPPH radical concentration by 50%. The lower the IC50, the higher the antioxidant ability of the extract (Erkan et al., 2008; Roby et al., 2013). The DPPH assay is based on DPPH-mediated oxidation, while the FRAP assay relies on the electron-donating capacity of bioactive compounds and the conversion of Fe$^{3+}$ to Fe$^{2+}$ (Karimi and Jaafar, 2011). The results of the FRAP assay were largely in agreement with those of the DPPH assay in this case, but an approach with more than one assay is highly advisable for obtaining reliable results (Schlesier et al., 2002; Aruoma, 2003; Ruberto et al., 2007). A positive correlation between the DPPH and FRAP methods has also been reported in other crops (Connor et al., 2002; Awika et al., 2003; Thaipong et al., 2006; Surveswaran et al., 2007; Tawaha et al., 2007; Ghahremani-majd et al., 2012). Antioxidant activity has been previously reported in tarragon plants (Piccaglia et al., 1993; Lopes-Lutz et al., 2008); however, the present study
was the first evaluation of the antioxidant capacities of several Iranian *A. dracunculus* accessions through comprehensive in vitro methods.

The results of the total phenolic and flavonoid contents were fairly well correlated to the DPPH and FRAP antioxidant assays, with tarragon accessions that had high phenolic and flavonoid contents exhibiting high antioxidant activity in both the DPPH and FRAP assays. Many other studies have also demonstrated a linear correlation between total phenolic content and antioxidant activity in plants (Tawaha et al., 2007; Wu et al., 2007; Pawar et al., 2011; Gahremani-majd et al., 2012). According to Tawaha et al. (2007), this positive correlation demonstrates that the phenolic compounds of *A. dracunculus* accessions contribute to their antioxidative effects. This correlation was expected because phenolic constituents are one of the most aqueous methanol-soluble antioxidants and can be present at high levels in herbs (Garcia-Salas et al., 2010). Consistent with the literature, we found that the total phenolic contents were highly correlated with the flavonoid contents in all tarragon accession extracts, which was expected as the flavonoids are phenolic compounds.

Plant polyphenols constitute one of the major classes of natural products used as primary antioxidants and anti-cancer compounds (Karimi and Jafar, 2011). In recent years, the health effects of dietary phenolics have come to the attention of researchers as well as food manufacturers (Dai and Mumper, 2010), and for this reason we assessed their content in the selected *A. dracunculus* accessions. Since total phenolic content estimated by the Folin-Ciocalteu method does not give an accurate insight into the quality and quantity of the phenolic compounds present in the extracts (Tawaha et al., 2007; Dai and Mumper, 2010), we utilized an RP-HPLC system to determine the individual phenolic constituents, which is the preferred technique for both the qualification and quantification of phenolic substances (Andrade et al., 1998; Santos-Buelga and Williamson, 2003; Khoddami et al., 2013). Because of the diversity and complexity of the natural phenolic compounds in plant extracts, the separation and identification of every compound can be difficult; however, identifying key phenolic compounds is simpler (Cai et al., 2004; Wojdylo et al., 2007). In the present study, the HPLC chromatogram of the tarragon extracts indicated that chlorogenic acid, syringic acid, and caffeic acid are the primary phenolic acids, while luteolin was the principal flavonoid compound present. The main coumarin was found to be herniarin. Several peaks in the chromatogram were not identified owing to the absence of pure standards. The phenolics identified in these Iranian *A. dracunculus* accessions have previously been reported in tarragon plants from other regions (Hofer et al., 1986; Onuchak et al., 2000; Saadali et al., 2001; Supilnikova, 2004; Lin and Harnly, 2012); however, the concentrations of these phenolics were found to vary between the accessions. Many studies have shown that the combined influences of genotypic and environmental factors largely affect antioxidant activities and phytochemical properties of crops (Faudale et al., 2008; Gahremani-majd et al., 2012; Karimi et al., 2015b). Karimi et al. (2015a, 2015b) revealed a high level of genetic, morphological, and phytochemical variations in the Iranian tarragon germplasm. Overall, the differences in antioxidant capacity and phenolic compounds reflect the variability of the tarragon accessions.

The tarragon accessions characterized in this study indicated a great deal of antioxidant activity and phenolic content variation. The high level of free-radical scavenging activity exhibited by the Birjand accession was interesting, as were its exceptionally high total phenolic and flavonoid contents. We have found that the Iranian tarragon accessions often present in Iranian dishes represent important dietary sources of phenolic antioxidants and could be used to replace the synthetic antioxidants added to various food products. Although a high-dose application of tarragon may pose health risks due to the mutagenic and hepatotoxic effects of estragol (Kalantari et al., 2013), the amount of this potentially harmful compound in tarragon extracts is limited. The consumption of tarragon extracts would, therefore, likely cause no significant side effects on the human body and has a great potential to enhance the quality of food and beverage products, as well as having possible applications in the pharmaceutical and cosmeceutical industries. Further scientific studies are needed to confidently support its use, however.

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