INTRODUCTION

Dandelion Taraxacum officinale (L.) Weber ex F.H. Wigg have been used as medicinal infusions and decoctions of root and herb (1). In Turkish folk medicine, the herb is used as a laxative, diuretic and potent anti-diabetic medicine (2). The traditional Chinese medicine uses Taraxacum officinale to enhance immune response to the upper respiratory tract infections, and bronchitis or pneumonia (1). In combination with other herbs, Taraxacum officinale is used to treat hepatitis. Their extracts contain lipotropic substances which can improve functionality of hepatocytes (3). Other several health-promoting benefits including anti-rheumatic, anti-inflammatory, anti-carcinogenic and hypoglycaemic activities have been attributed to the use of dandelion extracts or the plant itself (4,5) Taraxacum officinale flower extract, particularly the ethyl acetate fraction, possesses bioactive phytochemicals which have the ability to scavenge reactive oxygen species (ROS) and prevent DNA from ROS-induced damage in vitro (6). Dandelion can protect against cholecystokinin octapeptide induced acute pancreatitis in rats (7). In addition, leaves from Taraxacum officinale ingested by ruminants during pregnancy strengthen the liver and can help prevent preeclampsia, which manifests as high blood pressure with edema. Leaves help prevent anemia, because they are rich in iron and helps the fetus to develop a strong liver of its own (8). Also, the ethanolic extract of Taraxacum officinale increases the frequency and excretion ratio of fluids in healthy humans (9). Extracts from dandelion possess anti-influenza virus (10), anti-fertility (11) and showed strong anti-HIV–1 retrovirus activity (12), antioxidant and hepatoprotective effects (13). Chicoric acid is one of the main constituent of T. officinale. It is also known as dicaffeyltartaric acid and belongs to phenylpropanoids. Chicoric acid occurs naturally in large number of medicinal plants that were being used in folk medicine since time immemorial (14). Its anti-angiogenic, anti-inflammatory, antibacterial, anti-nociceptive activities and inhibit HIV integrase are well documented (12, 15, 16, 17).

MATERIALS AND METHODS

Plant material: Aerial part (leaves) of Taraxacum officinale was collected from Plovdiv Bulgaria. Extraction procedure: Samples of herbs desiccated in the lab at room temperature were pulverized in a laboratory crusher. The powder of ground sample (1.0 g) were carefully extracted with 100 ml of ethanol-water (1:1 v/v), 96 % ethanol and water in a water bath at 80°C for 1 h, stirred for 1 h at room temperature and then filtered. HPLC analysis: The HPLC analysis of phenolics acids were performed by Waters HPLC systems (Milford, MA, USA) equipped with binary pump (Waters 11525), a UV-VIS detector (Waters 2487) and Breeze 3.30 SPA software. Detailed conditions of HPLC analyses are previously reported by Marchev et al (18).
**Determination of total dihydroxycinnamic derivatives:**
Total dihydroxycinnamic acid contents (including caffeoyl derivatives) were expressed as chlorogenic acid as previously described in the European Pharmacopoeia (6th ed. 2008). The extract (1 ml) was added to 2 ml 0.5 M HCl, 2 ml Arnow’s reagent (10 g sodium nitrite and 10 g sodium molybdate made up to 100 ml with distilled water), 2 ml NaOH (at a concentration of 2.125 M) and 3 ml of water. Each solution was compared with the same mixture without Arnow’s reagent. Absorbance was read at 525 nm. The content of each plant was calculated and expressed as mg chlorogenic acid derivatives per g DW.

**Determination of total polyphenolic compounds:**
The total phenolic contents were measured using a Folin-Ciocalteu assay according to the procedure described by Stintzing et al. (20) with some modifications. Folin-Ciocalteu reagent (1mL) (Sigma) diluted five times was mixed with 0.2 mL of sample and 0.8 mL 7.5 % Na₂CO₃. The reaction was 20 min at room temperature in darkness. After reaction time, the absorption of sample was recorded at 765 nm against blank sample, developed the same way but without extract. The results were expressed in mg equivalent of gallic acid (GAE) per g dry weight (DW), according to calibration curve; build in range of 0.02 - 0.10 mg gallic acid used as a standard.

**Antioxidant activity (AOA):** 
**DPPH radical scavenging activity:** Investigated extract (150 μl) were mixed with 2850 μl freshly prepared DPPH solution (0.1 mM in methanol). The mixtures were incubated for 15 min at 37 °C in darkness and the reduction in absorbance at 517 nm was measured by spectrophotometer. A standard curve was created with Trolox in concentration between 0.005 and 1.0 mM. The results are expressed in mM Trolox® equivalents (TE) per g dry weight (DW).

**Ferric-reducing antioxidant power assay (FRAP):** FRAP assay was carried out by method described by Banzie and Strain (21) with some modifications. The reagent solution was freshly prepared by mixing acetate buffer (300 mM) 2,4,6-trpyridyl-s-triazine (TPTZ) solution (10 mM) : FeCl₃x6H₂O (20 mM) (in proportions of 10:1:1; v/v/v). 100 μl of investigated extracts were added to 3000 μl of reagent solution and allowed to react for 10 min at 37 °C. The absorbances of the formed colored product were measured at 593 nm. A standard curve was created with Trolox. The results are expressed in mM Trolox® (TE) per g DW.

**Cupric ion reducing antioxidant capacity:** The reaction was started by mixing of 1 ml CuCl₂xH₂O (10 mM in dd H₂O), 1 ml Neocuproine (7.5 mM in methanol), 1 ml ammonium acetate buffer (0.1 M; pH 7.0), 100 μl of investigated extract and 1 ml dd H₂O. The reaction time was 20 min at 50 °C. After cooling, the absorbance (450 nm) was read against a reagent blank, developed on the same way but the extract was replaced with methanol. A standard curve was created with Trolox. The results are expressed in mM Trolox® (TE) per g DW.
**RESULTS AND DISCUSSION**

The extraction yield is a measure of the solvent efficiency to extract specific components from the original materials. In the case of *T. officinale*, it will give an idea about the extractability of total phenolics including the caffeic acid derivatives under different extraction conditions. Therefore, in the present study, only the traditional solvent extraction was used to extract the phytochemicals in dandelion leaves. The established total polyphenolic compounds and total caffeic acid derivatives of *T. officinale* leaves were significantly affected by the ethanol concentration. The highest total polyphenolic concentration and total dihydroxycinnamic derivatives (33.90 ± 0.57 mg GAE/ g DW and 32.22 ± 0.22 mg CAE/ g DW, respectively) were registered by extraction with 50 % ethanol (Figure 1). The contents of total phenolics, individual and total caffeic acid derivatives in extracts were also affected by the ethanol concentrations (Table 1). These results are related to solvent polarity and solubility of compounds in tested solvents (22). In this study, when the ethanol volume percentage was lower than 50%, the contents of total phenols, cichoric acid, and total caffeic acid derivatives were increased with the increases of ethanol concentrations. However, the total phenols and total caffeic acid derivatives contents in extracts were decreasing when the ethanol concentration was 50%. This result is in agreement with the report of Tsai et al (23), that 50% ethanol is favorable for extracting individual caffeic acid derivatives from *E. purpurea*.

Phenolic acids are secondary metabolites that are commonly found in plant-derived foods. They have attracted considerable interest due to their many potential health benefits, which are powerful antioxidants and have been reported to demonstrate antibacterial, antiviral, anticarcinogenic, antiinflammatory, and vasodilatory actions (24).

The chemical composition of the phenolic acids of *T. officinale* in different extracts was determined by HPLC (Figure 2). As shown in Table 1, six components were identified (chlorogenic, caffeic, *p*-coumaric, sinapic, ferulic and cichoric acid). The most abundant phenolics acid in investigated samples were cichoric acid (484, 1195 and 3148 mg/100g DW) and sinapic acid (between 24-141 mg/100g DW) in various extracts of *T. officinale*.

*Echinacea purpurea* root and *Ocimum basilicum* L. have been reported to be a major human dietary sources of cichoric acid (25, 26). The yield of cichoric acid in thus plants between 1.0 g/100g DW – 2.0 g/100g DW by *E. purpurea* (25, 27), and *O. basilicum* between 52-88 mg/100g DW (26). *T. officinale* biosynthesized about 2 times more cichoric acid (3.1 g/100g DW) (Table 1) than *E. purpurea*, similar results for yield of cichoric acid obtain Fraisse et al. (19) – 3.4 g/100g DW. Amount of extracted cichoric acid from dandelion leaves with 50% ethanol was up to 89 % from total yield of extracted phenolics (Table 1). This results show that next purification and crystallization of cichoric acid from 50% ethanol extracts would be easily than extracts obtain from *E. purpurea*.

In recent years, interest in plant-derived food additives has grown. Plant extracts might substitute synthetic food antioxidants, which may influence human health when consumed chronically. Plant-derived food additives, especially polyphenolic compounds, have been ascribed health-promoting properties (28).

In our study, we decided to evaluate antioxidant activities of ethanol extracts of *T. officinale* by applying two methods, based on mixed [hydrogen atom transfer (HAT) and single electron transfer (SET)] mechanisms (DPPH) and two methods, based only on SET mechanism (FRAP and CUPRAC) (Table 2). Antioxidant activities of plant extracts were usually explained with the presence of phenolic acids in them (29). Thus, the 50 % ethanol extracts of *T. officinale* had the highest antioxidant activities and the highest total phenolics. Relatively high concentrations of cichoreic acid and sinapic acid in extracts of investigated plant were the most probable reason for observed high antioxidant activities in this plant. Table 2, presents data on the antioxidant activity of the extracts from *T. officinale* determined in vitro by DPPH radical scavenging activity and metal reducing activity. These results are expressed as mM Trolox® (TE) per g DW of total antioxidant capacity for each extract. Species that have the highest antioxidant activities are the richest in polyphenols and cichoric acid, while the least active species are less rich in polyphenols (Figure 1, Table 2). On the whole, the antioxidant activities of the extracts compared to their different polyphenolic levels showed good correlations between total phenolics or total dihydroxycinnamic derivatives and plant antioxidant capacity, i.e. $R^2 = 0.8951$ and $R^2 = 0.9977$, respectively, and a relatively very good correlation between chicoeric acid determined by HPLC and antioxidant activity, at $R^2 = 0.9992$ (DPPH method), $R^2 = 0.9867$ (FRAP method) and $R^2 = 0.9999$ (CuPRAC method). The established relationships it can be conducted that the presence of the cichoreic acid seemed to be the main factor dictating free radical scavenging and metal reducing activity of the extracts.

**CONCLUSION**

<table>
<thead>
<tr>
<th>Phenolic acids</th>
<th>Extraction with 95% ethanol</th>
<th>Extraction with 50% ethanol</th>
<th>Extraction with water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorogenic acid</td>
<td>18</td>
<td>37</td>
<td>N.D.</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>15</td>
<td>22</td>
<td>N.D.</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>12</td>
<td>88</td>
<td>N.D.</td>
</tr>
<tr>
<td>Sinapic acid</td>
<td>24</td>
<td>141</td>
<td>26</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>N.D.</td>
<td>97</td>
<td>12</td>
</tr>
<tr>
<td>Cichoric acid</td>
<td>484</td>
<td>3148</td>
<td>1195</td>
</tr>
</tbody>
</table>

*N.D. – not detected*
Table 2. Antioxidant activity in different extracts from T. officinale.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Radical scavenging activity</th>
<th>Metal reducing activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPPH EC 50, mg/ml</td>
<td>FRAP</td>
</tr>
<tr>
<td>95% ethanol extract</td>
<td>28.1 ± 0.8*</td>
<td>17.2</td>
</tr>
<tr>
<td>50% ethanol extract</td>
<td>136.3 ± 4.7</td>
<td>3.5</td>
</tr>
<tr>
<td>Water extract</td>
<td>54.1 ± 1.1</td>
<td>9.0</td>
</tr>
</tbody>
</table>

* - mM TE/ g DW

The current report is the first comprehensive study that presented detailed information for phenolic, content and antioxidant activity of edible dandelion (Taraxacum officinale (L.) Weber ex F.H. Wigg.) grown in Bulgaria. The antioxidant potential of extracts positively correlated with their phenolic contents and, respectively. Dandelion is important source of cichoric acid with potential application as radical scavengers and metal reducing activity. Therefore, this complex of biologically active substance offers many future applications in field of herbal medicine and nutrition for production of healthy food with well-pronounced healthy effect.

REFERENCES