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Assessment of Antioxidant and Cytotoxic Activities and Identification of Phenolic Compounds of *Centaurea solstitialis* and *Urospermum picroides* from Turkey

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HIGHLIGHTS

- The phenolic composition, antioxidant activity and cytotoxic potential of the extracts of *C. solstitialis* and *U. picroides* were investigated.
- Caffeic acid was found as the most abundant phenolic compound in the extracts.
- Both species showed promising antioxidant activity towards different assays.
- The highest cytotoxic potential was observed in the extract of *C. solstitialis*.

Abstract: It is known that some genera of the Asteraceae family are commonly used in Turkish folk medicine. Several studies have investigated the biological effects of different extracts of *Centaurea* and *Urospermum* species, but studies involving the phenolic composition of *C. solstitialis* and *U. picroides* extracts are very limited. This study aimed to investigate the phenolic composition and antioxidant activity of *C. solstitialis* and *U. picroides* and evaluate their possible cytotoxic effect. RP-HPLC analysis was used to elucidate the phenolic profiles of the ethanolic extracts of flowering parts of *C. solstitialis* and *U. picroides*. The both ethanolic extracts were assessed for their antioxidant properties using DPPH, FRAP, phosphomolybdenum and metal chelating assays. Furthermore, the effect of the extracts on cell viability was evaluated against MCF-7 and PC-3 cancer cells and HEK293 cell line using the MTT assay. The most abundant phenolic compound in both extracts was determined to be caffeic acid, and the amount of this compound was 24078.03

and 14329.59 $\mu\text{g g}^{-1}$ in the extracts of *C. solstitialis* and *U. picroides*, respectively. The antioxidant activity of the extracts was found similar. Compared with *U. picroides* extract, *C. solstitialis* extract had higher potential on the inhibition of cell viability. The IC_{50} value of *C. solstitialis* on MCF cells was found as 58.53 $\mu\text{g mL}^{-1}$. These data suggest that the extracts of *C. solstitialis* and *U. picroides* may be considered as novel and alternative natural antioxidant and anticancer sources.

Keywords: *Centaurea solstitialis*; *Urospermum picroides*; Antioxidant activity; Cytotoxic activity; HPLC.

INTRODUCTION

Turkey is one of the most floristically rich countries in the world with amazing plant diversity. Its flora consists of about 12000 vascular plants [1] and the therapeutic significance of many medicinal plants among them has not been recognized yet.

Centaurea L., which is a medicinal genus belonging to the Asteraceae family, is represented with 217 taxa 60% of which are endemic to Turkey [2]. Members of this genus were proposed to have several biological activities such as antioxidant [3,4], antimicrobial [5] and antiproliferative [6,7] effects. *Centaurea solstitialis* L. (yellow star thistle) which grows all over Turkey is used for herpes infections around the lips, malaria, upset stomachs, common colds, hemorrhoids, peptic ulcer and abdominal pain [8-11].

Urospermum picroides (L.) Scop. ex F. W. Schmidt (prickly golden fleece) is an annual herb of the Asteraceae family and used as a medicinal herb for the treatment of inflammatory diseases [12]. This species has been shown to have anti-inflammatory, antimicrobial, antioxidant and antiproliferative activities [12-14] and contain secondary metabolites such as sesquiterpene lactones and glycosides [15] and indole alkaloids [16].

The *Centaurea* and *Urospermum* genera represent a significant source of bioactive substances [13,17], but up to now, to the best of our knowledge, the phenolic compounds of the ethanolic extracts of *C. solstitialis* have not been clarified yet, and only one study has been performed with *U. picroides*' methanolic extract in Spain [18]. Therefore, we were inspired to manage this study to determine the phenolic compounds of the ethanolic extracts of *C. solstitialis* and *U. picroides* using RP-HPLC. Additionally, these extracts were evaluated for their potential antioxidant activities and cytotoxic properties against MCF-7 and PC-3 cancer cells and HEK293 cell line.

MATERIAL AND METHODS

Chemicals

The chemicals and reagents for the antioxidant and cell viability assays were purchased from Sigma-Aldrich (Germany), Merck (USA) and Biochrom (Germany).

Plant Materials and Extraction

C. solstitialis and *U. picroides* were collected in July and August, 2014 from Yerkesik, Muğla-Turkey (37°8'40" N, 28°17'11" E, 550 m) respectively. Taxonomic identification of the plants was confirmed by Dr. Fatma Güneş from the Department of Pharmaceutical Botany at Trakya University in Edirne, Turkey. All specimens were deposited in their herbarium (Voucher No: H.G. 902 and H.G. 901). The flowering parts of plants were air-dried (for one week) in shadow at 25 °C, and the dried flowers were powdered down to fine grains. Ethanolic extracts were produced using a Soxhlet extractor for 10 h. The extracts were filtered and vaporized by using a rotary evaporator and then lyophilized. The crude extracts were kept at -20 °C until needed. For the MTT assay, DMSO at 0.1% final concentration was used as solvent of the extracts. For the antioxidant assays, the extracts were solubilized in ethanol.

Total Phenolic and Flavonoid Contents

The phenolic and flavonoid contents of the plant extracts were determined by using the Folin-Ciocalteu protocol [19] and aluminum [20] colorimetric methods, respectively. These contents were expressed as gallic acid (mg GAEs g^{-1}) and quercetin (mg QEs g^{-1}) equivalents, respectively.

Phenolic Compound Analyses

Phenolic compounds of the extracts of *C. solstitialis* and *U. picroides* were analyzed by RP-HPLC (Shimadzu, Japan) as described by Caponio and coauthors [21] with some modifications. Separation was performed at 30 °C by using a reversed phase column (Agilent Eclipse XDB C-18, 250 mm × 4.6 mm, 5 µm) by a mixture of two solvents (A: the formic acid solution 3% and B: methanol) as a mobile phase. Gradient conditions were particularized at a flow rate of 0.8 mL min⁻¹ as follows: 93% A + 7% B for 3 min, 72% A + 28% B in 28 min, 67% A + 33% B in 60 min, 58% A + 42% B in 62 min, 50% A + 50% B in 70 min, 30% A + 70% B in 75 min, 93% A + 7% B in 80 min. Phenolic compounds in the ethanolic extracts of *C. solstitialis* and *U. picroides* are expressed as µg g⁻¹ extract, which were analyzed with a diode array detector (DAD).

Antioxidant Activity Assays

DPPH Radical Scavenging Activity

Extract solution (1 mL) was added to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical solution (0.004% methanol, 4 mL), and after 30 min of incubation at 25 °C in the dark, the absorbances were recorded at 517 nm. DPPH radical scavenging activity is expressed as Trolox (mg TEs g⁻¹ extract) equivalents [22].

Ferric Reducing Antioxidant Power (FRAP) Assay

Ferric reducing antioxidant power assay was applied as described by Zengin and Aktumsek [23] with some modifications. Extract solutions were added to FRAP reagent which was mixed in advance (acetate buffer– 0.3 mol L⁻¹, TPTZ (2,4,6-tripyridyl-s-triazine– 10 mmol L⁻¹, FeCl₃– 20 mmol L⁻¹). After measuring the absorbances at 593 nm, FRAP activity was expressed as Trolox (mg TEs g⁻¹ extract) equivalents.

Total Antioxidant Capacity (Phosphomolybdenum Method)

The phosphomolybdenum method was used to evaluate the total antioxidant capacity of the extracts. To keep it short, different extract solutions were mixed with the reagent solution (0.6 M H₂SO₄, 28 mmol L⁻¹ Na₃PO₄ and 4 mmol L⁻¹ (NH₄)₂MoO₄) and incubated for 90 min at 95 °C. The absorbance values were determined at a wavelength of 695 nm [24]. Total antioxidant capacity is expressed as Trolox (mg TEs g⁻¹ extract) equivalents.

Metal Chelating Activity

Extract solutions at different concentrations were added to FeCl₂ (0.05 mL, 2 mmol L⁻¹). The reaction that started directly after adding 5 mmol L⁻¹ of ferrozine was measured at 562 nm after being left for 10 min at room temperature. Metal chelating activity is expressed as EDTA (mg EDTAEs g⁻¹ extract) equivalents. [25].

Cell Cultures and MTT Assay

The cell lines were sourced from the American Type Culture Collection (ATCC, USA). MCF-7 (breast adenocarcinoma) (ATCC #HTB-22), PC-3 (prostate adenocarcinoma) (ATCC #CRL-1435) and HEK293 (human embryonic kidney) (ATCC #CRL-1573) cell lines were maintained in an RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 100 units mL⁻¹ penicillin and 100 µg mL⁻¹ streptomycin at 37°C in 5% CO₂. The cell viability was detected based on the MTT assay [26]. For this assay, cell lines were added separately at a final concentration of 4x10³ cells per well into 96-well plates as triplicates and incubated at 37°C for 24 h. Dose course experiments for 72 h were performed at seven different concentrations (1000-15.625 µg mL⁻¹) of each extract under the same conditions. The untreated cells were used as a control. At the end of this 72-h incubation, the medium with the extract was removed from the wells and replaced with 100 µL of fresh growth medium. Later, 10 µL of MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium Bromide) (5 mg mL⁻¹) solution to achieve a final concentration of 0.45 mg mL⁻¹ was added to every well, and the microplates were incubated for another 4 h. Then, the media containing MTT were discarded, and the formazan crystals formed by viable cells were dissolved in 100 µL of DMSO. The absorbance (Abs) for each well was recorded using a microplate reader at 540 nm. The percentage of cell viability according to the following formula:

$$\text{Cell viability \%} = (\text{Mean Abs of treated cells} / \text{Mean Abs of untreated cells}) \times 100 \quad (1)$$

Statistical Analysis

The results obtained in this study are expressed as mean \pm Standard error (SE). Statistical analysis and data processing were performed by using GraphPad Prism 7.0 (GraphPad Software, Inc., San Diego, CA). Comparisons of the treatments among groups were analyzed by one-way ANOVA with post-hoc Tukey's test. Significance was accepted as * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$, ns-no significance ($P > 0.05$). The IC_{50} values were also calculated using the GraphPad Prism software.

RESULTS

Total Bioactive Compounds and Phenolic Composition

Total phenolic and flavonoid contents of *C. solstitialis* and *U. picroides* ethanolic extracts were investigated with spectrophotometric methods, and the results are presented in Figure 1. According to the data obtained, the highest total phenolic content (52.31 mg GAEs g^{-1}) and total flavonoid content (30.10 mg QEs g^{-1}) were detected to be in the *C. solstitialis* extract. There were no significant differences between the two plants in terms of total flavonoid contents ($P > 0.05$), whereas a significant difference was found in terms of their phenolic contents ($P \leq 0.05$).

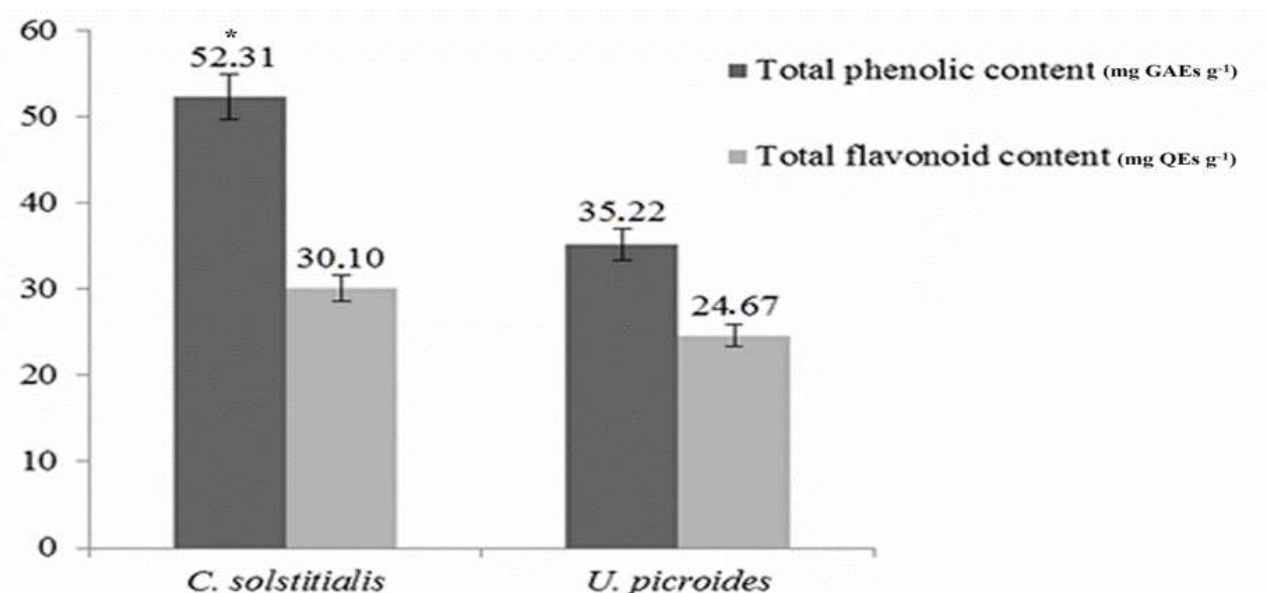
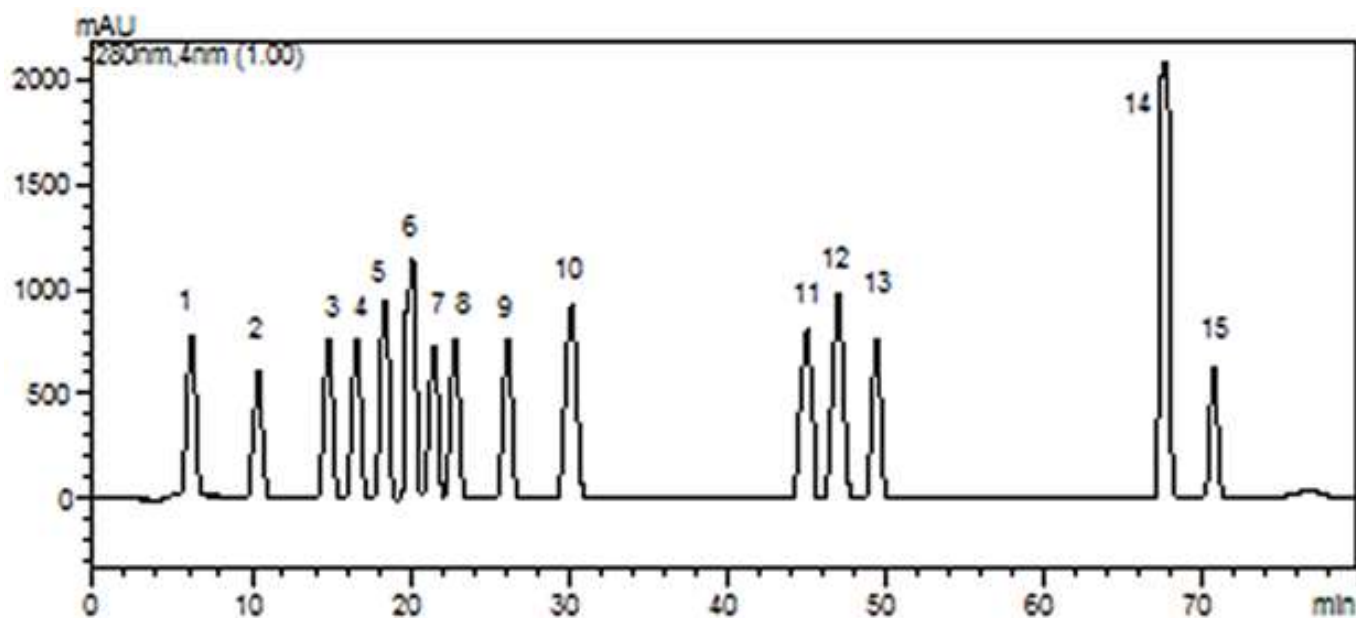


Figure 1. Total phenolic and flavonoid contents of *C. solstitialis* and *U. picroides* extracts. The bar represents the mean of contents (\pm SE) in each species. (GAEs: Gallic acid equivalents, QEs: Quercetin equivalents). * $P \leq 0.05$.

In order to identify the phenolic compounds in the ethanolic extracts of the flowering parts of both *C. solstitialis* and *U. picroides*, the RP-HPLC method was applied by using 15 phenolic compounds (gallic acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, chlorogenic acid, vanillic acid, epicatechin, caffeic acid, *p*-coumaric acid, ferulic acid, rutin, ellagic acid, naringin, cinnamic acid, quercetin) as the standards (Figure 2). The phenolic compounds were detected in both extracts (Figures 3 and 4) with varying amounts and are given in Table 1. Caffeic acid was detected to be the most abundant phenolic compound in both extracts, and the amount of this compound was 24078.03 and 14329.59 $\mu g g^{-1}$ extract in the *C. solstitialis* extract and the *U. picroides* extracts, respectively. Following caffeic acid, the main phenolic compounds of the *C. solstitialis* extract were determined as epicatechin (3684.22 $\mu g g^{-1}$ extract), 2,5-dihydroxybenzoic acid (3360.16 $\mu g g^{-1}$ extract) and 4-hydroxybenzoic acid (2077.33 $\mu g g^{-1}$ extract), respectively. The main phenolic compound in *U. picroides* extract was 4-hydroxybenzoic acid (1189.72 $\mu g g^{-1}$ extract) after caffeic acid. The amounts of epicatechin (499.33 $\mu g g^{-1}$ extract) and 2,5-dihydroxybenzoic acid (799.48 $\mu g g^{-1}$ extract) in the *U. picroides* extract were significantly lower than that of the *C. solstitialis* extract. *p*-coumaric acid was detected in both extracts as a minor compound.

Table 1. Phenolic compounds of *C. solstitialis* and *U. picroides* extracts ($\mu\text{g g}^{-1}$ extract) (mean \pm SE).

No	Phenolic Compounds	RT (min)	UV _{max} (nm)	LOD ($\mu\text{g mL}^{-1}$)	<i>C. solstitialis</i> ($\mu\text{g g}^{-1}$ extract)	<i>U. picroides</i> ($\mu\text{g g}^{-1}$ extract)
1	Gallic acid	6.8	280	0.01	92.59 \pm 0.95	168.43 \pm 2.06
2	3,4-dihydroxybenzoic acid	10.7	280	0.03	496.32 \pm 3.15	156.27 \pm 2.00
3	4-hydroxybenzoic acid	15.7	280	0.01	2077.33 \pm 43.20	1189.72 \pm 11.83
4	2,5-dihydroxybenzoic acid	17.2	320	0.75	3360.16 \pm 51.53	799.48 \pm 5.13
5	Chlorogenic acid	18.2	320	0.01	52.16 \pm 0.41	285.25 \pm 3.02
6	Vanillic acid	19.2	320	0.11	340.15 \pm 3.84	842.25 \pm 5.42
7	Epicatechin	21.3	260	0.43	3684.22 \pm 77.44	499.33 \pm 3.26
8	Caffeic acid	22.7	280	0.01	24078.03 \pm 495.5	14329.59 \pm 263.1
9	p-coumaric acid	26.1	320	0.01	41.00 \pm 0.33	2.14 \pm 0.08
10	Ferulic acid	30.1	320	0.01	127.21 \pm 1.64	23.27 \pm 0.52
11	Rutin	45.6	360	0.57	46.17 \pm 0.36	13.40 \pm 0.25
12	Ellagic acid	47.7	240	0.45	45.85 \pm 0.30	344.57 \pm 3.75
13	Naringin	49.7	280	0.40	120.62 \pm 1.48	13.76 \pm 0.36
14	Cinnamic acid	67.8	280	0.01	383.82 \pm 3.06	544.20 \pm 4.01
15	Quercetin	71.1	360	0.57	37.86 \pm 0.27	116.29 \pm 1.35

**Figure 2.** The HPLC chromatogram of standard phenolic compounds (1. gallic acid, 2. 3,4-dihydroxybenzoic acid, 3. 4-hydroxybenzoic acid, 4. 2,5-dihydroxybenzoic acid, 5. chlorogenic acid, 6. vanillic acid, 7. epicatechin, 8. caffeic acid, 9. p-coumaric acid, 10. ferulic acid, 11. rutin, 12. ellagic acid, 13. naringin, 14. cinnamic acid, 15. quercetin).

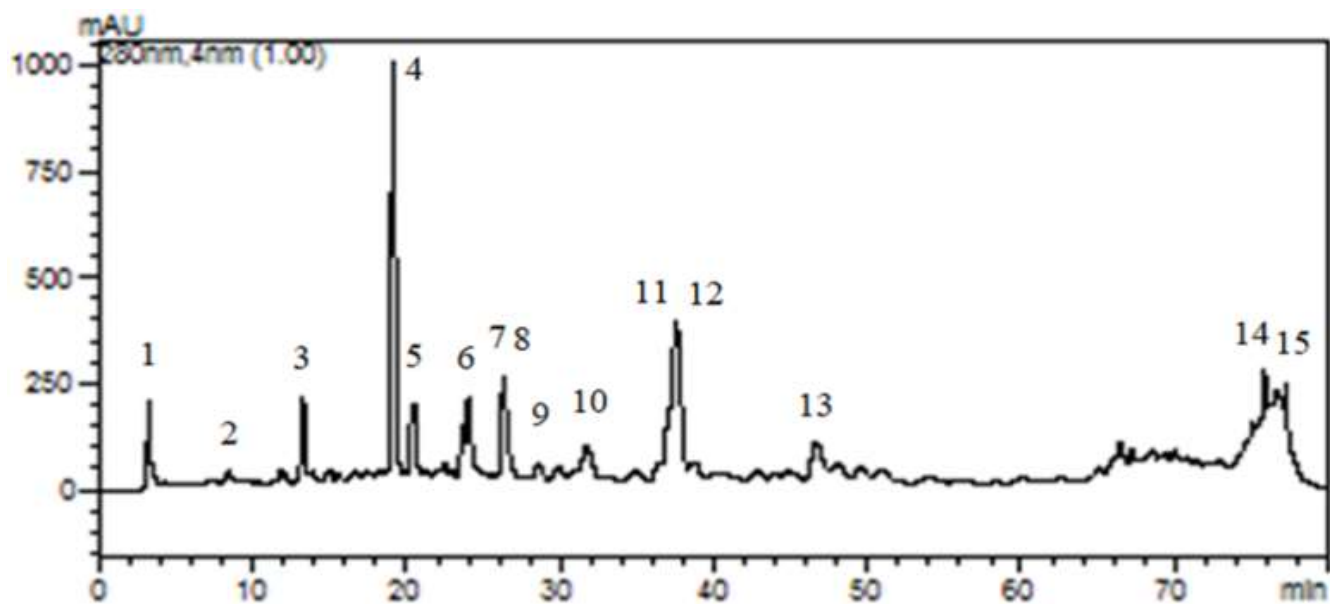


Figure 3. The HPLC chromatogram of *C. solstitialis* extract.

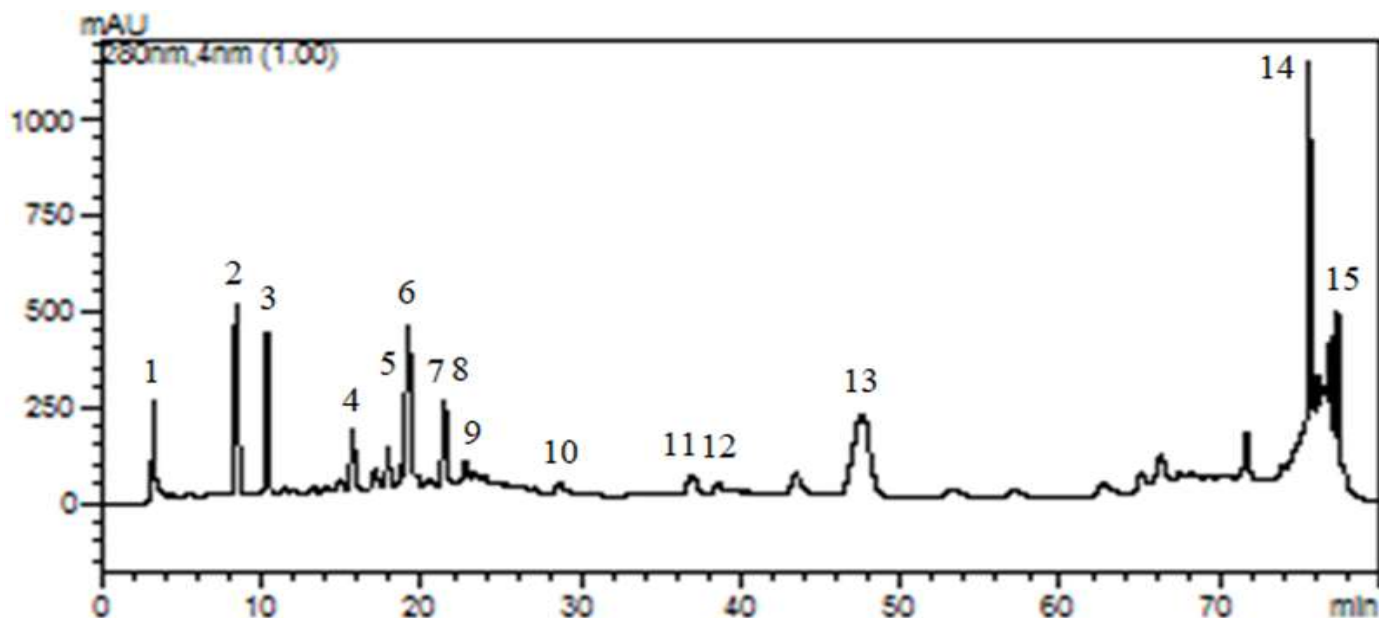


Figure 4. The HPLC chromatogram of *U. picroides* extract.

Antioxidant Activity

Four various antioxidant assays, DPPH, FRAP, phosphomolybdenum and metal chelating were applied to the ethanol extracts of the flowering parts of *C. solstitialis* and *U. picroides*. The outcomes of these assays are presented in Table 2. There were no significant differences between the two plants in terms of all the antioxidant assays that were applied ($P > 0.05$). However, the *C. solstitialis* extract showed a slightly higher antioxidant activity than *U. picroides*. The results of the DPPH, FRAP, phosphomolybdenum and metal chelating assays for *C. solstitialis* were determined as 55.04 mg TE g^{-1} , 65.45 mg TE g^{-1} , 49.23 mg TE g^{-1} , and 10.33 mg EDTAE g^{-1} extract, respectively.

Table 2. Antioxidant activities of *C. solstitialis* and *U. picroides* extracts (mean \pm SE).

Plants	DPPH (mg TEs g ⁻¹)	FRAP assay (mg TEs g ⁻¹)	Phosphomolybdenum assay (mg TEs g ⁻¹)	Metal chelating activity (mg EDTAEs g ⁻¹)
<i>C. solstitialis</i>	55.04 \pm 0.25	65.45 \pm 0.37	49.23 \pm 0.20	10.33 \pm 0.26
<i>U. picroides</i>	51.35 \pm 0.22	60.33 \pm 0.31	42.13 \pm 0.15	07.15 \pm 0.20

TEs: trolox equivalents, EDTAEs: EDTA equivalents.

Cytotoxicity of the Extracts

The effect of the extracts at different concentrations (1000-15.625 $\mu\text{g mL}^{-1}$) on viability of the cells tested for 72 h were determined by the MTT assay. The results of the MTT assay are graphically illustrated in Figure 5. The viability of the cancer cells (MCF-7 and PC-3) seemed to significantly decrease at higher concentrations than 125 $\mu\text{g mL}^{-1}$, when treated with both extracts separately. The MCF-7 cell viability was significantly decreased to about 90% when treated with the *C. solstitialis* extract in the concentration range between 1000 to 125 $\mu\text{g mL}^{-1}$ or the *U. picroides* extract at between 1000 to 250 $\mu\text{g mL}^{-1}$. The effects of both extracts on PC-3 cells were generally similar to each other. No cytotoxic activity of the *C. solstitialis* extract was found at 31.25 and 15.625 $\mu\text{g mL}^{-1}$ concentrations in MCF-7 cancer cells and HEK293 cells. However, the *C. solstitialis* extract at 62.5 $\mu\text{g mL}^{-1}$ had a lower cytotoxic potential against HEK293 cells in comparison to that of MCF-7 cells. The *U. picroides* extract at between 125 to 15.625 $\mu\text{g mL}^{-1}$ did not decrease the percentage of HEK293 cell viability. According to the approximate IC₅₀ values (50% inhibitory concentrations), the IC₅₀ values of both extracts were found to be lower in cancer cells than HEK293 cells. The IC₅₀ values of the *C. solstitialis* extract on MCF-7, PC-3 and HEK293 cells were 58.53, 91.47, 224.5 $\mu\text{g mL}^{-1}$, respectively. Moreover, the IC₅₀ values for the *U. picroides* extract were 115.5, 109.8 and 464.1 $\mu\text{g mL}^{-1}$ on MCF-7, PC-3 and HEK293 cells, respectively.

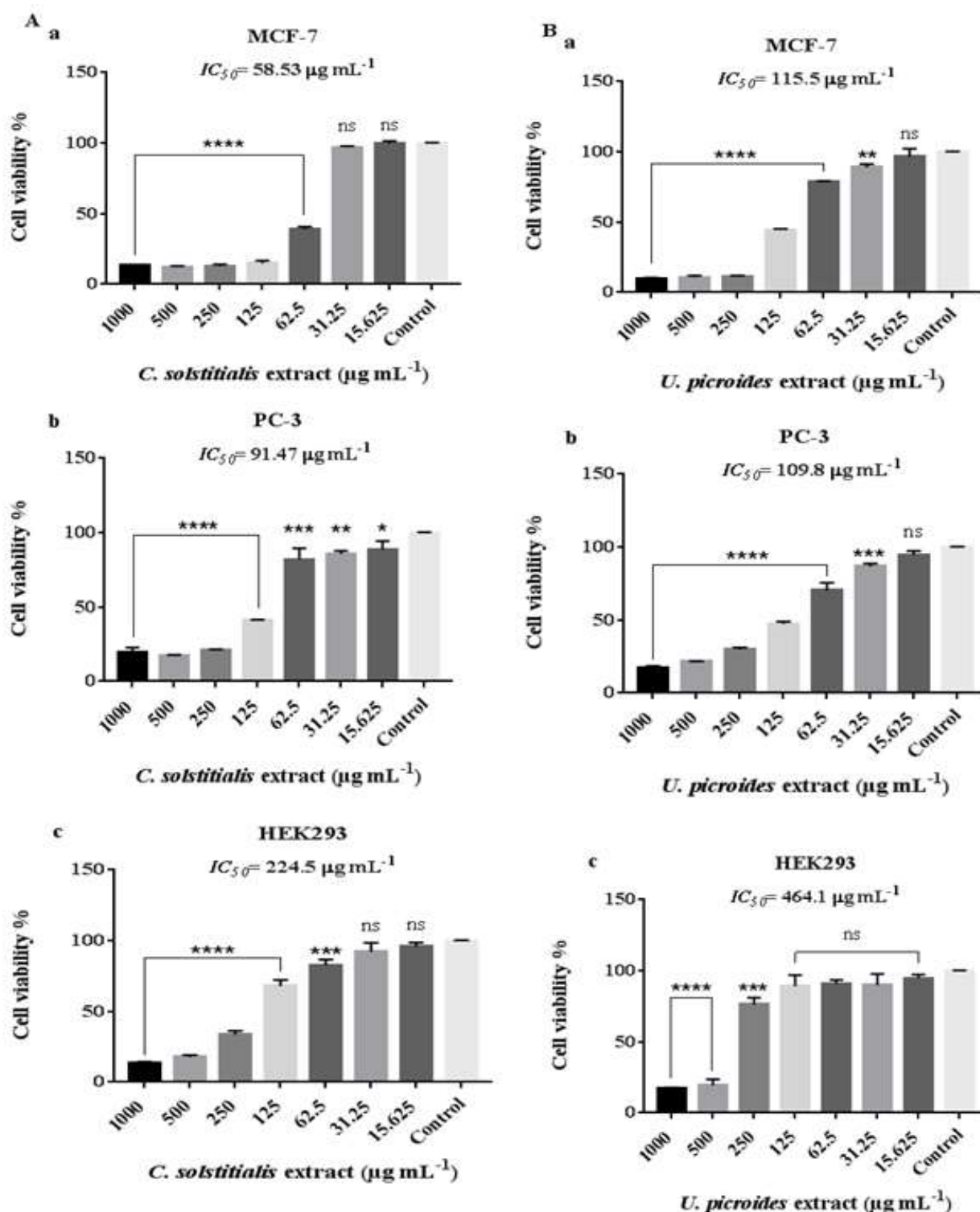


Figure 5. The effects of *C. solstitialis* (A) and *U. picroides* (B) extracts on cell viability. Each bar represents the percent of cell viability after treatment with different concentrations of the extracts. Data are means (\pm SE) of three independent experiments. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$, ns-no significance ($P > 0.05$).

DISCUSSION

Although many studies have been carried out on the evaluation of the antioxidant capacity of plants, a single method which completely reflects the antioxidant capacity has not been developed. Therefore, it is necessary to fully interpret antioxidant capacity using different chemical assays. From this point of view, different antioxidant assays (DPPH, FRAP, phosphomolybdenum and metal chelating) were used in our study, and the total phenolic and flavonoid contents were calculated for each extract.

In studies evaluating the antioxidant capacity of plants, at least one free radical was used, and the studies determined the rate at which this radical is scavenged by the plant extract. The most common of these

radicals is DPPH. Ferric reducing power assay (FRAP) is often used as an indicator of electron-donating activity, which is an important mechanism of antioxidant compounds, especially phenolics [27]. The phosphomolybdenum assay is based on reduction of Mo (VI) to Mo (V) and subsequent formation of a green phosphate/Mo (V) complex. Transition metals act as a catalyst for lipid peroxidation. Therefore, chelating these metals is considered as an important antioxidant mechanism [23].

Most phenolic compounds have been reported to possess potent antioxidant activity and have anticarcinogenic, anti-inflammatory and antibacterial activities to a greater or lesser extent [28,29]. A study was conducted by Cai and coauthors [30] examined the structure-radical scavenging activity relationships of phenolic compounds from anticancer-related traditional Chinese medicinal plants. The authors reported that hydroxycinnamic acids, such as caffeic acid and chlorogenic acid were found to be rather active in scavenging radicals related to antioxidant activity. The presence of abundant amounts of caffeic acid in both plant extracts examined in this study may explain the antioxidant activity of these plants.

Several researchers showed that the major phytochemical components of *Centaurea* taxa were sesquiterpene lactones, terpenoids, flavonoids and acetylenes [31,32]. Previous phytochemical studies showed that *Centaurea* taxa contain flavonoids including quercetin, luteolin, kaempferol, salvigenin, apigenin, hispidulin, cirsimaritin, apigenin 7-O-glucoside and isokaempferide [33,34].

Formisano and coauthors [35] reviewed reports on flavonoids from the Centaureinae subtribe of the family Asteraceae and reported that only 16 of 72 recognized genera of Centaureinae have been examined for the occurrence of flavonoids. According to these data, it was seen that the majority of the genera of the Centaureinae has not been examined for their flavonoid profile yet.

The results on the chemical composition of *C. solstitialis* reconfirmed the value of the *Centaurea* genus as a source of phenolic compounds. Our results were in accordance with the phytochemical profile of the *Centaurea* species from the Turkish flora [36,37]. The phenolic profile of the methanolic extract of *Centaurea urvillei* subsp. *stepposa* was obtained by using RP-HPLC analysis [36], and it showed a rich phenolic content.

Many earlier studies showed that the aerial parts of *Centaurea* species is an alternative source of phenolic compounds. Compared to other *Centaurea* species collected from Turkey, it was evident that the phenolic content of *C. solstitialis* is higher than the values found for the *Centaurea* species investigated by Şen and coauthors [38] (ranged from 4.82 to 12.46 mg GAEs g⁻¹) and lower than the values found for the *Centaurea* species investigated by Aktumsek and coauthors [39] (ranged from 82.27 to 175.40 mg GAEs g⁻¹). Koc and coauthors [40] reported that the total phenolic contents in the ethanolic extracts of the flowers and leaves of *C. solstitialis* were determined as 18.43 and 13.66 mg L⁻¹, respectively. It was reported that differences were observed in the phenolic contents of plant species belonging to the same genus depending on several factors including temperature, soil content and altitude (growing conditions) [41].

The results on total phenolic content in this study displayed a similar propensity to those of the antioxidant abilities. Accordingly, the high content of total phenolics in the extracts might explain the antioxidant properties of the extracts. These results were consistent with other results in the literature which demonstrated a strong relationship between antioxidant activities and total phenolic contents [23,42].

The DPPH radical scavenging, FRAP, metal chelating activities and the total antioxidant capacity of methanolic *Centaurea lycopifolia* extracts were reported previously as 52.49 mg TE g⁻¹, 64.44 mg TE g⁻¹, 8.63 mg EDTAE g⁻¹ and 1.45 mmol TE g⁻¹ extract, respectively [37]. In another study, the DPPH and FRAP activities of the methanolic *Centaurea urvillei* subsp. *stepposa* extracts were detected as 43.35 and 52.18 TEs g⁻¹ extract, respectively [36]. Based on these results, it may be concluded that *C. solstitialis* had a higher antioxidant activity than *C. lycopifolia* and *C. urvillei* subsp. *stepposa*. In addition, it was reported that DPPH free radical scavenging activity, reducing power activity and also total phenolic compound of *C. solstitialis* (from Konya) were evaluated [43].

EI-Amier and coauthors [44] detected the total phenolic and flavonoid contents of an aqueous extract of *U. picroides*, which was collected from Egypt, as 9.31 and 4.24 mg g⁻¹ dry weight, respectively. The authors also reported that the DPPH scavenging activity of *U. picroides* was found as 4.14% at 500 µg mL⁻¹. The total phenolic and flavonoid contents of methanolic *U. picroides* extracts were determined by EI-Amier and coauthors [14] as 19.34 and 8.28 mg g⁻¹ dry weight, respectively. According to these results, the ethanolic extract of *U. picroides*, which was collected from Turkey, had stronger antioxidant activity and more abundant total bioactive compounds than those collected from Egypt.

EI-Nabawy and coauthors [13] assessed the antioxidant activity of *U. picroides* using the ABTS method. Inhibition of ABTS free radicals were found to be 86.1% and 82.4% for the aerial parts in the ethyl acetate and methylene chloride fraction, respectively.

Giner and coauthors [18] reported that chlorogenic acid, quercetin, luteolin, quercetin-3-galactoside, kaempferol-3-galactoside, isochlorogenic acid and luteolin-7-glucoside were isolated from the methanolic extract of *U. picroides*, which was collected from Spain. The outcomes of the phenolic profile of *U. picroides*, which was studied in this study, were in good agreement with the aforementioned results.

Plant species and their bioactive compounds have an important role in inhibiting the progression of cancer and development of clinically useful new anti-cancer agents [45,46]. In our study, the ethanolic extracts from the flowering parts of both *C. solstitialis* and *U. picroides* were assessed on MCF-7, PC-3 cancer cells and HEK293 cells for 72 h. The *C. solstitialis* extract caused higher inhibition in the percentage of the viabilities of MCF-7 cells (IC_{50} value=58.53 $\mu\text{g mL}^{-1}$) and PC-3 cells (IC_{50} value=91.47 $\mu\text{g mL}^{-1}$) compared to HEK293 cells (IC_{50} value=224.5 $\mu\text{g mL}^{-1}$). The *C. solstitialis* extract was found to be more effective than the *U. picroides* extract (IC_{50} value = 115.5 $\mu\text{g mL}^{-1}$) in reducing viability of MCF-7 cells. The potential cytotoxic effects of both extracts on PC-3 cells were similar. Additionally, the IC_{50} value of the *U. picroides* extract against HEK293 cells was 464.1 $\mu\text{g mL}^{-1}$. Our results suggested that these extracts have more cytotoxic potentials against MCF-7 and PC-3 cancer cell lines than against HEK293 cell lines, in other words the cancer cell lines used herein were more sensitive to both extracts than HEK293 cell line. In contrast, the IC_{50} values of the ethanolic extract of the flowering parts of *C. solstitialis* on HeLa (cervix adenocarcinoma), Daudi (Burkitt's lymphoma, CCL-213), A549 (lung adenocarcinoma) and BEAS-2B (normal bronchial epithelium) human cell lines at 72 h were reported to be 63.18, 69.27, 252.5 and 75.25 $\mu\text{g mL}^{-1}$, respectively [7]. Erenler and coauthors [47] stated that, at 500 $\mu\text{g mL}^{-1}$, the methanolic extract of stem parts of *C. solstitialis* L. ssp. *solstitialis* collected from Tokat caused higher antiproliferative activity on HeLa cells than that of the methanolic extracts of root and flower parts of the plant, whereas all extracts at 1000 $\mu\text{g mL}^{-1}$ had about the same antiproliferative activity on C6 (rat brain tumor) cells. Among the different fractions of aerial parts and seeds of *U. picroides* from Egypt, the butanol fraction from seeds and the ethyl acetate fraction from aerial parts were indicated to be very strongly cytotoxic to both MCF-7 (IC_{50} value=9.4±0.37 and 8.8±0.47 $\mu\text{g mL}^{-1}$, respectively) and HePG-2 (liver carcinoma) (IC_{50} value=14.7±0.85 and 10.1±0.88 $\mu\text{g mL}^{-1}$, respectively) cell lines [13]. Furthermore, the IC_{50} values of the ethanolic extract from the flowering part of *U. picroides* were found to be 85.64 $\mu\text{g mL}^{-1}$ for Daudi, 135.35 $\mu\text{g mL}^{-1}$ for HeLa, 234.8 $\mu\text{g mL}^{-1}$ for A549 and 109.80 $\mu\text{g mL}^{-1}$ for BEAS-2B cell lines [48]. These results suggest that the region where the plant is collected, part of plant used, solvent chosen for extraction and cell lines that are tested may affect cytotoxic activity on different scales.

CONCLUSION

In conclusion, this study revealed the HPLC profile, antioxidant and cytotoxic potentials of ethanolic extracts of the flowering parts of *C. solstitialis* and *U. picroides*. Caffeic acid was found to be the highest amount in both extracts based on HPLC analysis, especially the *C. solstitialis* extract was a superb source of caffeic acid. Both extracts showed promising antioxidant potential towards different assays. The highest inhibitory effect on cell viability was obtained from the *C. solstitialis* extract in comparison to the *U. picroides* extract. It may be proposed that the biological activities of both species that were obtained may be ascribed to the presence of phenolic compounds. Our results showed that *C. solstitialis* and *U. picroides* may be accepted as a novel and alternative source of natural antioxidant and anticancer agents. These potential biological activities are needed to be approved *via* additional experiments. Future studies should focus on clarifying the substances responsible for the potential antioxidant and cytotoxic effects of the extracts. Also, detailed *in vivo* investigations may contribute to design of new drug or food additive formulations.

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