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Original article

Antioxidant Activities of Phenolic Compounds of *Centaurea ensiformis* P.H. Davis

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The aim of the present study was to investigate the antioxidant activities of 11 phenolic compounds previously isolated from an endemic *Centaurea ensiformis* P.H. Davis (Asteraceae) by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and ABTS decolorization assays. These compounds consisting of 2 phenolic glycosides (tacioside and protocatechuic acid), 2 acetophenon glycosides (picein and 4-hydroxyacetophenon 4-O-[6'-O-β-D-apiofuranosyl]-β-D-glucopyranoside), 1 coumarin glucoside (scopolin), 4 flavonoid glycosides (vicenin-2, schaftoside, neoschaftoside and chrysoeriol-7-O-rutinoside), 1 phenylpropanoid glycoside (syringin) and 1 neolignan glucoside (dihydrodehydrodiconiferyl alcohol 4-O-β-D-glucopyranoside). Protocatechuic acid and tachioside had the highest activity (DPPH IC_{50}: 6.47 and 22.87 mM; TEAC: 33.2±0.18 and 31.2±0.99, respectively) in both methods. Dihydrodehydrodiconiferyl alcohol 4-O-β-D-glucopyranoside also showed high activity compared to ascorbic acid in DPPH assay (IC_{50}: 27.7 mM). Our results concluded that the *C. ensiformis* have a potential source of antioxidants of natural origin.

Key words: *Centaurea ensiformis*, Antioxidant activity, DPPH, ABTS, Protocatechuic acid, Tachioside.

Centaurea ensiformis P.H. Davis'in Fenolik Bileşiklerinin Antioksidan Aktiviteleri

Bu çalışmanın amacı endemik *Centaurea ensiformis* P.H. Davis (Asteraceae)'den daha önce izole edilmiş olan 11 bileşiğin 2,2-difenil-1-pikrilhidrazil (DPPH) ve ABTS dekolorizasyon yöntemleri ile antioksidan aktivitelerinin incelenmesidir. Bu bileşikler 2 fenolik glikozit (taçiozit ve protokateşik asit), 2 asetofenon glikozidi (pisein ve 4-hidroksiasetofenon 4-O-[6'-O-β-D-apiofuranosil]-β-D-glukopiranozit), 1 kumarin glikozidi (skopolin), 4 flavonoit glikozidi (visenin-2, şaftozit, neoşaftozit ve krizoeriyol-7-O-rutinozit), 1 fenilpropanoit glikozidi (siringin) ve 1 neolignan glukozidinden (dihidrodehidrodikoniferil alkol 4-O-β-D-glukopiranozit) oluşmaktadır. Taçiozit ve protokateşik asit her 2 metotta da en yüksek aktiviteye sahiptir (DPPH IC_{50}: 6.47 ve 22.87 mM; TEAC: 33.2±0.18 ve 31.2±0.99, sırasıyla). Dihidrodehidrodikoniferil alkol 4-O-β-D-glukopiranozit de DPPH yönteminde askorbik asit ile karşılaştırıldığında yüksek aktivite göstermiştir (IC_{50}: 27.7 mM). Sonuçlarımız, *C. ensiformis*'in doğal kaynaklı bir antioksidan kaynağı olma potansiyelini ortaya koymaktadır.

Anahtar kelimeler: *Centaurea ensiformis*, Antioksidan aktivite, DPPH, ABTS, Protokateşik asit, Taçiozit

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INTRODUCTION

The genus *Centaurea* L. is distributed particularly in the South-Western, Central and Eastern regions of Turkey, represented by 192 taxa, 114 of which are endemic. (1). *Centaurea ensiformis* P.H. Davis (Asteraceae) is a Mediterranean endemic species localized in Sandras Mountain in South-West Anatolia (1) and listed as vulnerable in Turkish Red Data Book (2). It is a perennial plant, with erect up to 35 cm tall stems and tomenose leaves. The involucre is 28-35 mm high and 20-27 mm wide and ovoid. The florets are yellow and the pappus is 5-6 mm, smaller then achenes. It grows at *Pinus nigra* forests, 1700 m (1).

C. ensiformis has been previously evaluated for its secondary metabolites by our group and several different compounds have been isolated (3). GC-MS analysis of essential oil of the plant has shown to have carvacrol (17.4%), hexadecanoic acid (13.2%) and phytol (6.0%) (4) and hexane extract has shown to have caryophyllene oxide (28.72%), spathulenol (17.81%), eudesmol (13.03%) and β–bourbonene (8.51%) (5) as the main components. Methanol extract (1 mg/mL) of *C. ensiformis* has showed strong antioxidant activity with 86.19 ± 2.94 % FRSA and the total phenolic content of the extract have been reported as 59.33 ± 1.76 (6).

The search for antioxidants from natural sources has received much attention and efforts have been put into identify compounds that can act as suitable antioxidants to replace synthetic ones. Even though a variety of medicinal plants are known to have an antioxidant activities it remains unclear which of the compounds are the active ones. Therefore, research to identify antioxidative compounds is an important issue. These compounds could be isolated and then used as antioxidants for the prevention and treatment of free radical-related disorders.

The aim of the present study was to investigate the possible free radical scavenging activities and antioxidant capacities of previously isolated 11 pure compounds from methanolic extract of *C. ensiformis* by DPPH and ABTS assays. These compounds are; 2 phenolic glycosides (tacioside and protocatechuic acid), 2 acetophenon glycosides (picein and 4-hydroxyacetophenon 4-*O*-[6'-*O*-β-D-apiofuranosyl]-β-D-glucopyranoside), 1 coumarin glucoside (scopolin), 4 flavonoid glycosides (vicenin-2, schaftoside, neoschaftoside and chrysoeriol-7-*O*-rutinoside), 1 phenylpropanoid glycoside (syringin) and 1 neolignan glucoside (dihydrodehydrodiconiferyl alcohol 4-*O*-β-D-glucopyranoside (5).

EXPERIMENTAL

Plant material

Centaurea ensiformis P.H. Davis were collected from Mugla-Sandras Mountain, 1493 m, Turkey in June 2004. A voucher specimen was deposited in the Department of Pharmacetical Botany, Faculty of Pharmacy, IZEF Herbarium, Ege University, Turkey with number IZEF5672.

Extraction and isolation

Dried flowering aerial parts of the plant (870 g) was extracted with *n*-hexane, chloroform and methanol (3 x L) respectively and evaporated under reduced pressure to dryness. Methanolic extract (56.67g) was suspended in H_2O and partitioned with *n*-BuOH successively. BuOH layer was evoporated to afford a residue (13.61 g) which was subjected to isolation process using column chromatography. The structures of the compounds were characterized by 1D- and 2D-NMR experiments and mass spectoscopy analysis (5).

Chemicals

DPPH, ascorbic acid, methanol, chloroform, n-hexane, potassium persulphate, Trolox, ABTS were analytical grade and obtained from the Sigma-Aldrich Chem, Steinheim, Germany.

DPPH radical scavenging activity

Free radical scavenging activity (FRSA) of the compounds on stable was determined spectrophotometrically and calculated as a percentage of 2,2-diphenyl-1-picrylhydrazyl (DPPH) discolouration. The DPPH assay was performed as previously described (7) with simple modifications. Briefly, pure compounds in methanol (100 µL) at different concentrations (1, 5, 10, 25, 50 and 100 µg/mL) were added to 200 µM DPPH in methanol and mixed in 96 well microplate. After a gentle mixing and standing 30 min at room temperature, the absorbance of the

resulting solution was measured at 517 nm in microplate reader. Ascorbic acid was used as positive control.

The percent DPPH scavenged by each pure compound was calculated using the following equation:

% Inhibition= $[(A_B-A_A)/AB]x100$

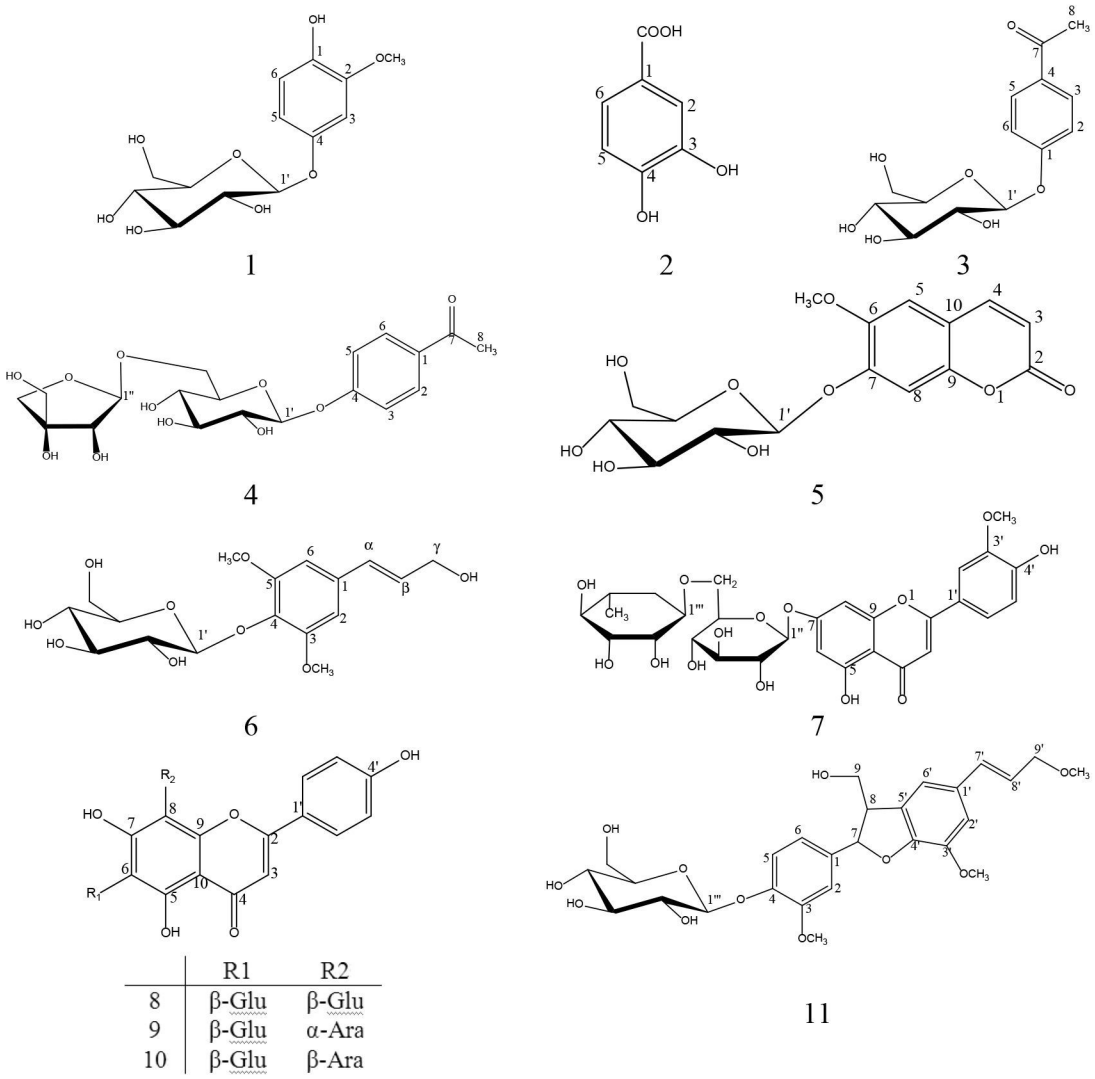
A_B: Absorbance of control (t: 0 min)

A_A: Absorbance of sample (t: 15 min)

Ascorbic acid concentrations were 62.5, 100, 166.67, 250 and 500 μg/mL (Prepared by dilution from 1 mg/mL stock solution).

Trolox equivalent antioxidant capacity assay (TEAC)

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) cation was generated in dark at room temperature by reacting a 7 mM solution of ABTS with 2.45 mM potassium persulphate (final concentration) for 24 h. The resulting $ABTS^+$ was diluted with 5 mM phosphate buffer (pH:7.4) to give an absorbance reading of 0.700 ± 0.02 at 734 nm. 10μL



	R1	R2
8	β-Glu	β-Glu
9	β-Glu	α-Ara
10	β-Glu	β-Ara

Figure 1. Chemical structures of the tested compounds; tacioside (1), protocatechuic acid (2), picein (3), 4-hydroxyacetophenon 4-O-[6'-O-β-D-apiofuranosyl]-β-D-glucopyranoside (4), scopolin (5), syringin (6), chrysoeriol-7-O-rutinoside (7), vicenin-2 (8), schaftoside (9), neoschaftoside (10), dihydrodehydrodiconiferyl alcohol 4-O-β-D-glucopyranoside (11).

Table 1. DPPH free radical scavenging activity of compounds of *C. ensiformis* (µg/mL)

Compound	1	5	10	20	50	100	IC_{50} (µM)
Tacioside	9.2	27.2	52.9	83.2	93.9	95.2	22.87
Protocatechuic acid	45.1	86	94.6	95.4	95.4	94.6	6.47
Picein	-	-	1.1	6.8	11.8	31.3	533
4-hydroxyacetophenon 4-*O*-[6'-*O*-β-D-apiofuranosyl]-β-D-glucopyranoside	-	-	-	-	3.2	23.6	-
Scopolin	-	-	-	-	-	1.1	-
Syringin	10.2	21	40.5	65.5	93.6	95	36.04
Vicenin-2	5.5	12.9	21.2	40.4	75.7	93.62	53.22
Schaftoside	-	-	-	3.8	27.8	23.3	-
Dihydrodehydrodiconiferyl alcohol 4-*O*-β-D-glucopyranoside	9.3	21.7	35.9	56.9	76.5	92.5	27.7
Neoschaftoside	-	-	2.7	9.3	18.5	45.4	198
Chrysoeriol-7-*O*-rutinoside	-	-	-	1.4	3.4	20.7	-
Ascorbic acid	-	-	-	-	-	-	20.5

Table 2. Trolox equivalent antioxidant capacity of compounds of *C. ensiformis* (mM).

Compound	TEAC
Tacioside	31.2±0.99
Protocatechuic acid	33.2±0.18
Picein	-
4-hydroxyacetophenon 4-*O*-[6'-*O*-β-D-apiofuranosyl]-β-D-glucopyranoside	9.1±0.07
Scopolin	-
Syringin	-
Vicenin-2	-
Schaftoside	-
Dihydrodehydrodiconiferyl alcohol 4-*O*-β-D-glucopyranoside	5.7±0.40
Neoschaftoside	6.5±0.03
Chrysoeriol-7-*O*-rutinoside	19±0.54

sample (1mg/mL), was reacted with 1 mL of $ABTS^+$ solution and absorbance (A) measured at 734 nm after 6 min (9).

TEAC of each pure compound was calculated using the following equation:

% Inhibition = $(A_{ABTS}+ - A_{6.\,min}) \times 100 / A_{ABTS.+}$

$A_{ABTS.+}$: Absorbance of $ABTS^+$ at 734 nm (0.700 ± 0.02)

$A_{6.\,min}$: Absorbance of $ABTS^+$ at 6. min after addition of samples

All measurements were performed three times. Trolox equivalency of the samples was calculated by comparing with a standard curve prepared with Trolox. Trolox concentrations were 2.5 mM, 5 mM, 7.5 mM, 10 mM and 15 mM.

RESULTS AND DISCUSSION

In the present study, DPPH radical scavenging activity and ABTS decolorization assays were used to evaluate the antioxidant activity of the phenolic compounds previously isolated from *C. ensiformis* (Figure 1). Stable free radical species such as $DPPH^•$ and $ABTS^{•+}$ are often used for the evaluation of the general radical scavenging capabilities of various antioxidants (7). As seen in Table 1, the highest DPPH radi-

cal scavenging activity was observed with protocatechuic acid (IC_{50}:6.47μM) and tacioside (IC_{50}:22.87 μM) when compared with ascorbic acid (IC_{50}: 20.5 μM).

High TEAC of both compounds was also found as 31.2±0.99 and 33.2±0.18 mM, respectively (Table 2). Because of antioxidant capacity of benzoic acid derivatives can be related to hydroxyl group numbers, protocatechuic acid was found more active than tacioside. Protocatechuic acid is a well-known antioxidant (8) and found in *C. ensiformis* fractions with high amounts therefore it could be responsible for high antioxidant activity of the plant extract (5). Dihydrodehydrodiconiferyl alcohol 4-*O*-β-D-glucopyranoside has also shown a remarkable DPPH radical scavenging activity (IC_{50}:27.7 μg/mL) relative to ascorbic acid.

A phenylpropanoid syringin is a glucoside of sinapyl alcohol, which was previously reported with antioxidant activity (IC_{50}: 85 μM) with DPPH method (10) showed higher activity (IC_{50}: 36.04 μM) in our study. Vicenin-2 is an apigenin 6,8-di-*C*-β-D-glucopyranoside. Vuciks et al. reported the DPPH radical scavenging activity of vicenin-2 as IC_{50}: 52.97 μg/mL and TEAC as IC_{50}: 17.86 μg/mL (11). These results noted as modarete activity when compared to positive control were found compatible with our results. Among the flavon *C*-diglycosides, schaftoside and neoschaftoside has lower antioxidant capacity when compared with vicenin-2 in both methods might be due to having fewer OH groups related to arabinose moiety. Results of chrysoeriol-7-O-rutinoside are consistent with previous report (12). Notable activity was not observed with acetophenon glycosides and the coumarin glucoside skopolin, by our study.

Free radicals play an important role in various pathological conditions such as tissue injury, inflammation process, neurodegenerative diseases, cancer and aging. The compounds that can scavenge free radicals have great potential in ameliorating these disease processes. Antioxidants thus play an important role to protect the human body against damage by reactive oxygen species (13). Among the important constituents participating in the cell defence system against free radicals are phenolic compounds (14). Phenolics have received increasing attention because of some interesting new findings regarding their biological activities. Our results clearly revealed that the antioxidant activity of the methanolic extract of *C. ensiformis* which has been previously reported is due to antioxidant phenolic content. Determination of the naturally occurring antioxidant compounds from bioactive plant extracts will help to develop new drug candidates for antioxidant therapy.

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REFERENCES

1. Davis PH, Mill RR, Flora of Turkey, Volume 5, Edinburgh, pp 582, Edinburgh University Press, Edinburgh, 1988.
2. Ekim T, Koyuncu M, Vural M, Duman H, Aytaç Z, Adıgüzel N, Red Data Books on Turkish Plants, Barıscan Press, Ankara, 2000.
3. Baykan Erel Ş, Bedir E, Khan IA, Karaalp C, Secondary metabolites from *Centaurea ensiformis* P.H. Davis, Biochem Syst Ecol 38(5), 1056-1058, 2010.
4. Karamenderes C, Demirci B, Baser KHC, Composition of essential oils of ten *Centaurea* L. taxa from Turkey, J Ess Oil Res 20(4), 342-349, 2008.
5. Ugur A, Duru ME, Ceylan O, Sarac N, Varol O, Kivrak I, Chemical composition, antimicrobial and antioxidant activities of *Centaurea ensiformis* Hub.-Mor. (Asteraceae), a species endemic to Mugla (Turkey), Nat Prod Res 23(2), 149-67, 2009.
6. Karamenderes C, Konyalioglu S, Khan S, Khan IA, Total phenolic contents, free radical scavenging activities and inhibitory effects on the activation of NF-kB of eight *Centaurea* L. species, Phytother Res 21(5), 488-491, 2007.
7. Brand-Williams W, Cuvelier ME, Berset C, Use of a free radical method to evaluate antioxidant activity, Lebensm Wiss Technol 28, 25-30, 1995.
8. Zhang Z, Liao L, Moore J, Wu T, Wang Z, Antioxidant phenolic compounds from walnut

kernels (*Juglans regia* L.), Food Chem 113, 16-165, 2009.
9. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Rad Biol Med 26, 1231-1237, 1999.
10. Es-Safia NE, Kollmanna A, Khlific S, Ducrota PH, Antioxidative effect of compounds isolated from *Globularia alypum* L.structure–activity relationship, LWT-Food Sci Tech 40, 1246-1252, 2007.
11. Vukics V, Kery A, Bonn GK, Guttman A, Major flavonoid components of heartsease (*Viola tricolor* L.) and their antioxidant activities, Anal Bioanal Chem 390, 1917-1935, 2008.
12. Delazar A, Sabzevari A, Mojarrab M, Nazemiyeh H, Esnaashari S, Nahar L, Razavi SM, Sarker SD, Free-radical-scavenging principles from *Phlomis caucasica,* J Nat Med 62, 464-466, 2008.
13. Pala FS, Gürkan H, The role of free radicals in ethiopathogenesis of diseases, Adv Mol Biol 1, 1-9, 2008.
14. Rohman A, Riyanto S, Yuniarti N, Saputra WR, Utami R, Mulatsih W, Antioxidant activity, total phenolic, and total flavonoid of extracts and fractions of red fruit (*Pandanus conoideus* Lam), Int Food Res J 17, 97-106, 2010.

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