



Antioxidant Activities of *Heracleum platytaenium* extracts and Essential Oil

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ABSTRACT

Heracleum platytaenium Boiss. has been used in traditional medicine. Antioxidant effect of essential oil as well as extracts of *H. platytaenium* was investigated. The essential oil was acquired by hydro distillation using a Clevenger type apparatus and GC-MS was used to analyze the essential oil compounds. Antioxidant capacity including ABTS⁺, DPPH[•] scavenging and reducing power activity tests were carried out for essential oil and extracts. Moreover, total phenolic and total flavonoid contents were investigated. *n*-Octyl acetate (36.5%), apiol (24.9%), and elemicin (20.8%) were the chief products of essential oil. The essential oil and extracts exhibited from weak to moderate activity. The total phenol varied from 19.01 to 130.99 mg GAE/g extract and total flavonoid was fluctuated from 2.0 to 118.4 mg QE/g extract. The most DPPH[•] scavenging effect was observed in EtOAc extract (IC₅₀ = 24.09 mg ml⁻¹). The ABTS⁺ scavenging effect of EtOAc extract was better than synthetic antioxidants BHA, BHT and Trolox.

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Heracleum platytaenium Ekstraktlarının ve Uçucu Yağının Antioksidan Aktiviteleri

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ÖZ

Heracleum platytaenium Boiss. geleneksel tıpta kullanılmaktadır. *H. platytaenium*'un uçucu yağının ve ekstraktlarının antioksidan aktivitesi incelenmiştir. Uçucu yağ, Clevenger cihazı kullanılarak su buharı damıtma yoluyla elde edildi ve bileşenler GC-MS ile belirlendi. Uçucu yağ ve ekstraktlar için ABTS⁺, DPPH[•] ve indirgeme gücü aktivitesi yöntemleri kullanılarak antioksidan kapasiteleri belirlendi. Ayrıca bitkinin toplam fenolik ve toplam flavonoid içerikleri araştırıldı. *n*-Oktil asetat (%36,5), apiol (%24,9) ve elemisin (%20,8) bileşikleri uçucu yağın ana ürünleridir. Uçucu yağ ve ekstraktlar zayıf ile orta derece antioksidan aktivite sergilemişlerdir. Toplam fenolik bileşik 19,01 ile 130,99 mg GAE/g ekstre arasında ve toplam flavonoid 2,0 ile 118,4 mg QE/g ekstre arasında değişiklik göstermiştir. En yüksek DPPH serbest radikal giderme etkisi EtOAc ekstresinde gözlenmiştir (IC₅₀ = 24,09 mg ml⁻¹). EtOAc ekstresinin ABTS⁺ radikal giderme etkisi sentetik antioksidan olan BHA, BHT ve Trolox'dan daha yüksektir.

Introduction

Natural products have gained the great interest due to the revealing the considerable biological activities (Erenler et al., 2016a; Erenler et al., 2016b; Erenler et al., 2016c; Karan and Erenler, 2017a; Karan and Erenler, 2017b; Karan et al., 2018a).

The genus of *Heracleum* belonged to Apiaceae family. It contained almost 125 species distributed around the world. This genus is represented in Turkey flora by 23 species, 9 of which are endemic (Pimenov and Leonov 2004). Most *Heracleum* species have been consumed in folk medicine in many countries (Bahadori et al 2016). *Heracleum* species have been used for remedy of different diseases such as anticonvulsant (Sayyah et al 2005), antihypertensive (Gao et al., 2014), anti-inflammatory (Yang et al., 2002), external tumor (Sathak et al., 2014), antifever (Karimi and Ito 2012). The root of *Heracleum* has also been applied in folk medicine as analgesic (Taniguchi et al., 2005), antipyretic (Taniguchi et al., 2011). Furthermore, these species have been employed in food as spices and flavoring agent (Souri et al., 2004). Phytochemical investigation on *Heracleum* species led to the isolation of active compounds basically coumarins, furocoumarins dimers, coumarin glycosides, anthraquinones, and stilbenes (Dincel et al., 2013). *H. platytaenium* essential oil displayed the antibacterial, antifungal (Akcin et al., 2013), anticandidal (Iscan et al., 2004) activities. The environmental factors, genetic heritage, abiotic stress, phenological stages of plants lead to the diversity in the chemical contents of essential oils (Bayir et al., 2014). Therefore, some differences in essential oil compounds and quantities were found between this result and literature (Kılıç et al., 2016).

Antioxidants have been extensively applied as food ingredients to protect foods against oxidative deterioration. Free radicals cause many ailments including ageing process, acute liver toxicity, inflammation process, diabetes, cancer, heart disease, Alzheimer, and Parkinson (Aruoma 2003). Due to the toxicity of synthetic antioxidant, aromatic and medicinal plants extract as well as active compounds have been investigated to be as a natural antioxidant recently (Erenler et al., 2017).

Essential oils (EOs) are aromatic oily liquids produced from plant material. Due to the revealing a great deal of biological effect, EOs have been broadly applied in food, cosmetic and pharmaceutical industries. EOs play a significant role in the preserving of the plants against enemies. EOs may also attract some insects to help the distribution of pollens and seeds or reject unwanted ones (Baby et al., 2010).

Although some researches were executed on antioxidant activity of *Heracleum* species such as *H. sprengeianum* (Sathak et al., 2014), *H. transcaucasicum*, *H. anisactis* (Torbatı et al., 2014), *H. nepalense* (Dash et al., 2005) *H. persicum* (Coruh et al., 2007), any research was not reported for *H. platytaenium* on antioxidant activity.

Due to the importance of this genus for pharmaceutically and medicinally as well as including bioactive compounds. In addition, essential oil of *Heracleum* species revealed a great deal of biological activities, we aimed to assess the antioxidant activities of *Heracleum platytaenium* essential oils and extracts.

Materials and Methods

Plant Material

H. platytaenium was collected from Artvin, Turkey in July 2017 and it was characterized morphologically by Prof. Dr. Ozgur Eminagaoglu, Department of Forestry Engineering, Faculty of Forestry, Artvin Coruh University.

Extraction

After dried and powdered, the aerial parts (100 g) of the plant were extracted with hexane (3×200 mL), ethyl acetate (3×200 mL) and methanol (3×200 mL) sequentially for 3 days at room temperature. The same plant material was used for each extraction. After removal of solvent under reduced pressure, hexane, ethyl acetate and methanol extracts were yielded.

Isolation of Essential Oil

The air-dried of *H. platytaenium* (250 g) was applied to hydro-distillation for 3 hours using a Clevenger-type apparatus. The distilled essential oil was stored (+4°C) for further usage.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Dilution of essential oil was executed with acetone (1:10) and Perkin-Elmer Clarus 500 model Auto system GC-MS, and BPX5 column (30 m × 0.25 mm × 0.25 µm film) were used. The temperature of column was kept initially at 50°C for 3 min and then raised by 5°C/min up to 220°C. Helium gas was utilized as a mover gas at 1mL/min flow rate with split ratio (50:1). The detector and injection temperature were 250°C (Karan et al., 2018b). 70 eV ionization energy was applied for detection.

Identification of the Compounds

Essential oil constituents were identified by mass spectra and comparing of their retention times with those of actual compounds or by checking of their retention index to *n*-alkanes series.

Determination of Antioxidant Activity

DPPH Free radical scavenging assay: The DPPH free radical scavenging activities of *H. platytaenium* extracts, essential oil and standards were tested by 1,1-diphenyl-2-picryl-hydrazil radical (DPPH[•]). Briefly, DPPH[•] was prepared (0.26 mM) in ethanol. DPPH[•] solution (1.0 mL) was added to the various concentrations of samples (3 mL, 2.5-20 µg/mL). The reaction mixture was stirred at room temperature for 30 min (Blois 1958). The absorbance measurement was executed at 517 nm on a spectrophotometer and lower absorbance of the reaction product showed the higher activity. The DPPH[•] scavenging activity was calculated by the given formula:

$$\text{DPPH (\%)} = (\text{Acontrol} - \text{Asample}) / \text{Acontrol} \times 100$$

ABTS^{•+} Scavenging assay: The principle of this method is connected with the capacity of clearing the ABTS^{•+} [2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)] radical cation by antioxidants. ABTS^{•+} was developed by treatment of ABTS (2.0 mM) in water with

potassium persulfate ($K_2S_2O_8$) (2.45 mM), stored at room temperature for 4h in the dark. ABTS⁺ was diluted to adjust the absorbance as 0.750 ± 0.0025 at 734 nm with sodium phosphate buffer (0.1 mM, pH 7.4). Afterward, ABTS⁺ solution (1.0 mL) reacted with each sample solution (3.0 mL) at different concentrations (2.5, 5.0, 10, 20 $\mu\text{g/mL}$). 30 min later, the inhibition (%) was calculated at 734 nm for each concentration comparative to a blank. The decolorization rate was calculated as absorbance of reduction percent. The calibration curve was used for calculation of ABTS⁺ concentration. The following equation was applied for the capability of ABTS⁺ scavenging effect (Re et al., 1999).

$$\text{ABTS (\%)} = (A_{\text{initial}} - A_{\text{remaining}}) / A_{\text{initial}} \times 100$$

Ferric reducing antioxidant power assay (FRAP): The reducing capability of essential oil and extracts were presented (Re et al., 1999). The samples at different concentrations in ethanol (1.0 mL) were added to a buffer of sodium phosphate (1.25 mL, 0.2 M, pH 6.7) and $[K_3Fe(CN)_6]$ (1%, 1.25 mL). The reaction flask was incubated for 25 min at 50°C. Iron (III) chloride (0.1% 0.25 mL) and CCl_3COOH (10%, 1.25 mL) were added to the reaction flask which was vortexed. The values of absorbance were recorded at 700 nm. High absorbance amount indicates high reducing capacity.

Total Phenolic Content

Folin-Ciocalteu (FC) was used to find out the total phenolic amount in extracts. GA was applied for calibration curve. The absorbance measurement was carried out at 765 nm on a spectrophotometer (Hitachi U-2900) (Slinkard and Singleton 1977). Each extract solution (0.1 mL, including 1000 μg extract) was diluted with distilled water (4.5 mL). FC reagent (0.1 mL) was added to the reaction flask of each extract solution. After 3 min, Na_2CO_3 (0.3 mL, 2%) was supplemented and reaction mixture was kept for 2 h with mixing.

Total Flavonoid Content

The contents flavonoid of extracts was identified by aluminium trichloride method applying quercetin as reference compound. Each extract (0.125 mL) was treated with $NaNO_2$ (5%, 0.075 mL). After allowing standing for 6 min, aluminum trichloride (0.15 mL, 10%) was added to the reaction mixture and incubated for 5 min then sodium hydroxide (0.75 mL, 1.0 M) and distilled water was added until the mixture to be 2.5 mL. The reaction mixture was incubated for 15 min then the absorbance was recorded (510 nm) (Zhishen et al., 1999).

Statistical Analysis

ANOVA (SPSS 12 for Windows) was used for variance analysis and three parallel experiments were carried out. P values <0.05 were regarded as significant.

Results

Composition of the Essential Oils

The essential oil composition was identified of GC-MS analyses. *n*-Octyl acetate (36.5%), apiole (24.9%), and elemicin (20.8%) were found as the chief compounds of the corresponding essential oil (Table 1).

Table 1 Chemical constituent of the essential oil of *H. platytenium*

| No | Compounds | RT* | RI** | Area (%) |
|-------|-----------------------------|-------|------|----------|
| 1 | Heptanal | 5.91 | 883 | 0.18 |
| 2 | α -Pinene | 6.62 | 988 | 0.16 |
| 3 | octanal | 9.44 | 1002 | 0.46 |
| 4 | (Z)- β -Ocimene | 10.51 | 1034 | 0.33 |
| 5 | (E)- β -Ocimene | 10.94 | 1045 | 0.43 |
| 6 | <i>n</i> -Octanol | 12.37 | 1075 | 7.69 |
| 7 | <i>n</i> -Nonanal | 13.72 | 1081 | 0.23 |
| 8 | (3Z)-3-Octenyl acetate | 17.74 | 1097 | 0.22 |
| 9 | <i>n</i> -Octyl acetate | 18.42 | 1185 | 36.47 |
| 10 | Octyl propionate | 22.37 | 1284 | 0.33 |
| 11 | Nonyl acetate | 22.70 | 1292 | 0.40 |
| 12 | α -Copaene | 25.15 | 1353 | 0.21 |
| 13 | <i>n</i> -Octyl butyrate | 25.94 | 1375 | 0.85 |
| 14 | Decyl acetate | 26.70 | 1395 | 0.22 |
| 15 | Octyl 2-methylbutanoate | 27.42 | 1429 | 0.27 |
| 16 | α -Curcumene | 28.96 | 1471 | 0.93 |
| 17 | Zingiberene | 29.37 | 1495 | 0.52 |
| 18 | β -Bisabolene | 29.74 | 1509 | 0.67 |
| 19 | δ -Cadinene | 30.05 | 1524 | 0.34 |
| 20 | β -Sesquiphellandrene | 30.24 | 1537 | 0.92 |
| 21 | Myristicin | 30.55 | 1538 | 0.25 |
| 22 | Elemicin | 31.34 | 1540 | 20.80 |
| 23 | Octyl hexanoate | 31.88 | 1574 | 0.87 |
| 24 | (-)- δ -Cadinol | 33.72 | 1631 | 0.22 |
| 25 | Apiole | 34.63 | 1679 | 24.86 |
| 26 | Octyl octanoate | 36.52 | 1767 | 0.27 |
| 27 | Hexahydrofarnesyl acetone | 37.88 | 1842 | 0.28 |
| Total | | | | 99.37 |

*RT: Retention times (min), **RI: Retention indices calculated against *n*-alkanes, % calculated from FID data.

Antioxidant Activities of Plant

The antioxidant capacities of *H. platytenium* extracts and essential oil were determined by DPPH[•], ABTS⁺ scavenging and FRAP assays using BHA, BHT, and Trolox as standards. In addition, quantification of phenolic and flavonoid (Table 2) were presented.

The DPPH[•] scavenging activity of ethyl acetate extract displayed the best activity among the extracts with the value of 24.09 (IC₅₀, $\mu\text{g/mL}$). In concern to the ABTS⁺ scavenging activity, EtOAc extract displayed the outstanding effect (IC₅₀, 3.98 $\mu\text{g/mL}$) compared to the standard Trolox (5.35 $\mu\text{g/mL}$). The considerable ABTS⁺ scavenging activity was not observed for the essential oil (47.91 $\mu\text{g/mL}$), hexane extract (35.90 $\mu\text{g/mL}$), and methanol extract (17.06 $\mu\text{g/mL}$). In reducing power assay, EtOAc extract showed the good activity. However, essential oil, hexane extract and methanol extract did not present the considerable effect as well.

The flavonoid contents of the extracts in terms of quercetin (QE) equivalent were between 2.04 ± 0.12 and 118.39 ± 1.23 . The total phenolic contents in the extracts of *H. platytenium* were expressed in terms of gallic acid (GA) equivalent, which is a common reference compound. The total phenol varied from 19.01 ± 0.35 to 130.99 ± 1.53 mg g⁻¹.

Table 2 Antioxidant activity and total flavonoid, phenolic contents of *H. platytaenium*

| Samples and standarts | Total flavonoid (mg QE/g ext) | Total phenolic (mg GAE/g ext) | DPPH* scavenging [IC ₅₀ (µg/mL)] | ABTS ⁺⁺ scavenging [IC ₅₀ (µg/mL)] | Reducing power (µmol TE/g ext) |
|-----------------------|-------------------------------|-------------------------------|---|--|--------------------------------|
| Essential oils | nt | nt | 1096.18±14.61 | 47.91±2.52 | 0.16±0.02 |
| Hexane extract | 2.04±0.12 | 19.01±0.35 | 355.02± 3.64 | 35.90 ± 0.68 | 0.28 ± 0.04 |
| EtOAc extract | 118.39±1.23 | 130.99±1.53 | 24.09 ± 0.50 | 3.98 ± 0.09 | 1.96 ± 0.02 |
| MeOH extract | 22.05±0.52 | 28.34±0.45 | 93.25 ± 0.90 | 17.06 ± 1.02 | 0.88 ± 0.05 |
| Trolox | nt | nt | 5.77 ± 0.11 | 5.35 ± 0.05 | nt |
| BHA | nt | nt | 4.89 ± 0.15 | 4.85 ± 0.18 | 5.46 ± 0.17 |
| BHT | nt | nt | 7.65 ± 0.16 | 5.75 ± 0.23 | 3.92 ± 0.11 |

Values represent the mean of the average of three experiments ± SD, nt: not tested.

Discussion

In this study, twenty-seven compounds (which accounted for 99.37% of total constituents in oil) from aerial parts of *H. platytaenium* were determined by GC-MS. *p*-Cymene (33.9%), terpinolene (14.3%), γ -terpinene (7.1%), elemicine (3.1%) and myristicine (2.9%) were found as major products for the roots of *H. platytaenium* essential oil (Kılıç et al., 2016). Elemicine was detected as a chief constituent of *H. platytaenium* essential oil in our work. The difference between present study and the others may be on account of the fact that we examined the whole aerial parts. *H. sphondylium* subsp. *ternatum* essential oil had weak DPPH, ABTS⁺ and FRAP effect compared with Trolox and BHT (Maggi et al., 2014).

In this study, the highest DPPH⁺, ABTS⁺ and FRAP activities of EtOAc extract could be attributed to the phenolic and flavonoid compounds. EtOAc extract included the most phenolics and flavonoids among the extracts. Therefore, functional groups in phenol and flavonoid displayed the excellent antioxidant effects such as giving the hydrogen and electron to the radicals. In comparison of hexane and methanol extracts, methanol extract exhibited the better activities than hexane extract. Methanol extract consisted of more phenolic and flavonoid than that of the hexane extract. Essential oil revealed the weak activity. This could be due to the compounds in essential oil having less ability to scavenge the radicals.

Antioxidant features are very important in protecting against free radical damage in biological systems and food (Cheung et al., 2007). The DPPH is a reliable free radical scavenging, which indicate activities of antioxidants. The lower IC₅₀ value detects a more powerful capacity of the extract to behave as a DPPH scavenger (Pirbalouti et al., 2014).

Antioxidant activities of four *Heracleum* species have been examined using the DPPH⁺ assay. *H. pastinacifolium* and *H. persicum* essential oils revealed the high activities with IC₅₀ values of 7.3 and 7.4 mg/ml, respectively (Firuzi et al., 2010). Fruit of *H. aquilegifolium* showed effective antioxidant activity compared to standard BHA and BHT (Karuppusamy and Muthuraja 2010).

Several secondary metabolites such as coumarins, lignans and flavonoids were isolated from the genus *Heracleum* (Park et al., 2010, Walasek et al., 2015, Xiao et al., 2005). The flavonoids are highly effective antioxidants with the ability to modulate the activity of various receptors and their interaction with specific receptors (Erenler et al., 2014). *H. persicum* fruits

displayed the weak DPPH⁺ scavenging activity. Also, the total flavonoid content was indicated as 22.23 µg rutin equivalents/g extract (Nickavar and Abolhasani 2009). Phenols also exhibit antioxidant properties due to their hydroxyl groups, which acidic protons can easily be donated (Hatano et al., 1989). In a study of some species of *Heracleum*, the total phenolic content was reported to be equivalent to 0.390 to 1.809 mg catechin/g oil (Firuzi et al., 2010).

Conclusion

The EtOAc extract of *H. platytaenium* exhibited the greatest antioxidant activity. This could be attributed to the phenolic and flavonoid compounds found in the extract. *H. platytaenium* has a potency to be a natural antioxidant. In addition, it could be used in pharmaceutical and food industries. Essential oil has a potency to be used in cosmetic and perfumery due to the including high amount of essential oil of *H. platytaenium*. The secondary metabolites of this plant should be isolated and identified to find novel bioactive agents. This plant includes the bioactive compounds which may be applied in medicinal purpose. There is direct proportion between the phenolic, flavonoid content and antioxidant activity.

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