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Comparison of Biological and Antioxidant Activities of Above and Below-Ground Extracts of Endemic *Heliotropium samolifolium* subsp. *erzurumicum*

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ABSTRACT

In this study, antioxidant, antimicrobial activities and effects on DNA damage of above and below-ground extracts of *Heliotropium samolifolium* subsp. *erzurumicum* were determined. This subspecies distributes only in the vicinity of Olur (Erzurum) in Turkey and is known as Erzurum Bambulu by the people. *Heliotropium* L. species include secondary metabolities such as; pyrrolizidine alkaloids, terpenoids, saponins, phenols, flavonoids, tannis, and steroids. The above and below-ground extracts of the subspecies were prepared using different organic solvents. For the antioxidant studies, DPPH and total phenolic content calculation methods were applied. The antimicrobial activity tests of the extracts were performed using four different standard strains, a yeast and MIC (Minimum Inhibition Concentration) method. The effects on DNA damage of plant extracts were explained using pBR322 plasmid DNA. The below-ground ethanol extract of the subspecies was seen to have stronger antimicrobial activity. According to antioxidant data, the highest activity was found in above-ground ethanol+aqueous, chloroform and below-ground ethanol extracts. Also, the below-ground aqueous and chloroform extracts had a greater effect on the open ring form of pBR322 plasmid DNA. It was determined that the below-ground extracts of the subspecies were more effective than the above ground extracts. It was suggested that the extracts obtained from this subspecies may be used in medicine industry and folk medicine.

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Endemik *Heliotropium samolifolium* subsp. *erzurumicum*'un Topraküstü ve Toprakaltı Ekstraklarının Biyolojik ve Antioksidan Aktivitelerinin Karşılaştırılması

ÖZET

Bu çalışmada, *Heliotropium samolifolium* subsp. *erzurumicum*'un topraküstü ve toprakaltı ekstraklarının antioksidan, antimikrobiyal aktiviteleri ve DNA hasarı üzerine etkileri belirlendi ve karşılaştırıldı. Bu alttür Türkiye de sadece Olur (Erzurum) çevresinde yayılış göstermektedir ve halk tarafından Erzurum Bambulu olarak bilinmektedir. *Heliotropium* L. türleri pirolizidin alkoloitleri, terpenoitler, saponinler, fenoller, flavonoitler taninler ve steroitler gibi sekonder metabolitleri içermektedir. Alttürün topraküstü ve toprakaltı ekstrakları farklı organik çözücüler kullanılarak hazırlandı. Antioksidan çalışmalar için DPPH ve toplam fenolik içerik hesaplama metotları uygulandı. Ekstrakların antimikrobiyal aktivite testleri dört farklı standart suş, bir maya mantarı ve MIK metodu kullanılarak yapıldı. Bitki ekstraklarının DNA hasarı üzerindeki etkileri pBR322 plazmit DNA'sı kullanılarak açıklandı. Bu alttürün toprakaltı etanol ekstraklarının daha güçlü antimikrobiyal aktiviteye sahip olduğu görüldü. Antioksidan sonuçlara göre, en yüksek aktivite topraküstü kloroform, etanol+su ve toprakaltı etanol ekstraklarında bulundu. Ayrıca toprakaltı su ve kloroform ekstrakları pBR322 plazmit DNA'nın açık halkasal formu üzerinde daha büyük bir etkiye sahiptir. Alttürün toprakaltı ekstraklarının topraküstü

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ekstraklarından daha etkili olduğu belirlendi. Bu alttürden elde edilen ekstrakların ilaç endüstrisinde ve halk hekimliğinde kullanılabileceği önerildi.

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INTRODUCTION

Turkey has a rich flora in terms of biodiversity in Europe. Medicinal and endemic plants in this rich flora constitute an important place. A large number of medicinal plants in Turkey have widely been used in many fields such as; tea, spices, dyes, ornaments, smell, taste industry, perfumes, cleaning, food and cosmetics by the people for many years (Başer, 2000; Toroğlu and Çenet, 2006). Nevertheless, medicinal plants have different effects on bacteria, fungi and viruses depending on the chemical structure and concentration of contain compounds. Thus, these plants have been a source of hope for many years in the treatment of many diseases caused by microorganisms.

In many studies, it has been reported that *Heliotropium* L. species contain various secondary metabolites such as saponins, tannins, steroids, terponoids, flavanoids, phenols and pyrrolizidine alkaloids (Singh et al., 2002; Goyal and Sharma, 2014; Santhosha et al., 2015; Roy, 2015). In particular, the pyrrolizidine alkoloids are one of the major secondary metabolites of this genus and have been identified more than 200 alkaloids. These alkaloids are extremely toxic and demonstrate anticancer activity and cytotoxic effects (Sharma et al., 2009; Singh and Sharma, 2019). Since secondary metabolites isolated from *Heliotropium* species have antimicrobial, antitumor, antiviral, anti-inflammatory, wound healing, cytotoxic and phytotoxic effects, these species have been used extensively in folk medicine, inflammation, gout, rheumatism, skin diseases (wart and rash), menstrual disorders, eye diseases**,** ulcer, febrile diseases, burns and poisonous animal bites for a long time(Singh et al., 2002; Reddy et al., 2002; Shoge et al., 2011; Ghaffari et al., 2013; Dash and Abdullah, 2013; Mourin et al., 2013**;** Yasmin, 2014; Ahmad et al., 2015; Roy, 2015). In particularly, the methanol and ethanol extracts of *H. indicum* have an important effect on the healing of wounds caused by *S. aureus* and *P. aeruginosa* (Yasmin, 2014).

Pyrrolizidine alkaloids, saponins, tannins and triterpenoids in *Heliotropium* species were found to be responsible for antimicrobial activities (Scott and Osho, 2012). At the same time, antimicrobial activities of isolated pyrrolizidine alkaloids and triterpenoids from *H. ellipticum* Ledeb, *H. subulatum* Hochst. ex DC. and *H. filifolium* (Miers) Reiche were proved, respectively (Jain and Sharma, 1987; Jain et al., 2001; Singh et al., 2002; Urzua et al., 2008).

Heliotropium is one of the important xerophytic and major genera of the Boraginaceae family. The vegetative diversity of the genus is seen in the widely different habitats and environments, and the species of genus spread out in tropical, subtropical, arid, semi-arid regions, on dry soils, gypsum hills, eroded slopes and warm-temperate areas of world (Diane et al., 2002). The genus includes perennial and annual herbs, subshrubs or rarely shrubs (Riedl, 1978). Annual and perennial species are generally distributed in the mountains and deserts, very dry habitats, respectively. This genus is represented by 17 species in Turkey and more than 300 species in the world (Diane et al., 2002; Akhani, 2007; Luebert et al., 2011; Güner et al., 2012). 4 of the natural spreading taxa (*H. ferrugineogriseum* Nabelek*, H. haussknechtii* Bunge*, H. samolifolium* Bunge subsp. *erzurumicum* Dönmez and *H. thermophilum* Kit Tan, A. Çelik & Gemici) in Turkey are endemic (Güner et al., 2012). The *Heliotropium* genus is easily distinguished from allied genera of the family by its scorpioid cymes and highly modified stigma heads that are very different from the rest of the taxa of this family (Kandemir et al., 2020). *H. samolifolium* subsp. *erzurumicum* is herbaceous, annual, 10-50 cm high, dense villous hairy, inflorescence with 10-50 sessile flowered. The subspecies is known as "Erzurum Bambulu" by people and is generally distributed at altitudes of 900-930 m and in metamorphic rocky (Dönmez, 2008). Because of distribution only around Olur (Erzurum), it is among the endemic plants have a limited distribution in Turkey.

The aim of this study was to determine and compare the antioxidant, antimicrobial activities and effects on plasmid DNA of above and below-ground extracts of the subspecies. In addition, the antioxidant and antimicrobial aspects of this plant is to provide its usefulness in different fields consciously.

MATERIAL and METHODS

Collection and Identification of Plant Samples

Plant samples were collected from flowering periods (July and August) from metamorphic rocks around Buzluca Village between Olur and Yusufeli, which is the natural distribution area. The taxonomic description of the subspecies was made according to Dönmez (2008). The above and below-ground parts of the fresh plant samples were divided into small pieces and dried in the shade on the benches in the laboratory. Then, these dried plant samples were

milled using a mill and used for biological activity studies.

Preparation of Plant Extracts

The above and below-ground parts of the plant were extracted with Soxhlet apparatus in the presence of different organic solvents (hexane, chloroform, ethyl acetate, ethanol, ethanol+aqueous and aqueous). For experimental studies, 50 gr of plant samples were weighed, put into soxhlet cartridges and extracted with hexane, chloroform, ethyl acetate, ethanol, ethanol+aqueous and aqueous for 8 h. After, the organic solvents evaporated by evaporation apparatus and the resulting plant extracts were stored at -20°C until analyzed.

Antimicrobial Activity

Antimicrobial activity studies were performed according to Minimum Inhibitory Concentration method (MIC) (Andrews, 2001). Microorganisms were obtained from Ondokuz Mayıs University. Gram positive (*Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* NRLLB1018), Gram negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) standard bacterial strains and a yeast (*Candida albicans* ATCC 10231) were used. Stock solutions of the used extracts were prepared at a concentration of 40 mg ml-1. Extracts were dissolved in DMSO. In broth dilution method, cultures were released to grow in 5 ml nutrient broth at 37°C for 18 h in 175 rpm shaker incubators.1 ml nutrient broth containing microorganisms was added to the test tubes. The compounds were added to the appropriate concentrations and half-serial dilution was performed. Tubes with serial dilution were allowed to incubate at 37°C in the incubator for 24 h. The last tube without bacterial growth was determined as MIC value. MIC values obtained in the study were shown as µg ml-1 (Table 1).

DPPH Free Radical Scavenging Activity

The antioxidant activities of the above and below-ground extracts were tested by DPPH free radical scavenging activity. Butillated hydroxi anisole (BHA) was used as standard antioxidant. For this process, 50 µl of different concentrations (3-10 mg ml-1) of plant extracts were incubated with 2850 µl of DPPH solution ($6x10^{-5}$ M) in the dark and at room temperature for 30 minutes. At the end of this process, the absorbance was measured at 517 nm against the blank sample (Brand-Williams et al.,1995). DPPH % was calculated according to formula (I). The results were expressed as IC_{50} value (Table 2). IC_{50} value demonstrates to the concentration of plant at the moment when half of the DPPH amount is scavenging.

Inhibition %= $(A_{DPPH}-A_{sample})/A_{DPPH}$ x 100

Respectively, A_{DPPH} refers to the DPPH radical in the absence of plant extract and A_{sample} refers to the DPPH radical in the presence of plant extract absorbance (at 517 nm).

Total Phenolic Content Activity

The amount of phenolic contents of the above and below-ground extracts were determined according to the method reported by Singleton and Rossi (1965). Gallic acid was used as the standard phenolic compound. Stock solution was prepared by dissolving 1 mg gallic acid in 1 ml organic solvent (methanol). 10, 25, 50, 75 and 100 µl from stock solution were received and transferred to test tubes. The final volume was completed to 2400 µl with pure methanol. After, 50 µl of Folin-Ciocalteu reagent was added. Then, 150 µl from 2% (w/v) Na_2CO_3 solution was added to test tubes and incubated for two hours at room temperature. The absorbance of the samples was read at 760 nm against the blank, which did not contain a test sample. The results were determined as µg(GAE)ml-1(extract) (Table 3).

DNA Interaction

The effects on plasmid DNA of the above and below-ground extracts of plant were determined by agarose gel electrophoresis method (Babu et al., 2007). Initially, 1% agarose gel was prepared in TBE (1X) buffer. 120 µg ml-1 plant extracts were interacted with 0.5 µg ml-1 pBR322 plasmid DNA at 37^0C for 2 h. After incubation, samples were mixed with 6X loading dye and loaded on 1% agarose gel. The electrophoresis was carried out at 100 v for 80 min. Then, gel was stained with EtBr (Ethidium Bromide) and the bands were imaged. Photographs were taken under UV light. The results were expressed and interpreted as the percentage of fragmentation of the DNA forms.

RESULTS and DISCUSSION

Plants have the ability to produce a large number of secondary metabolites. Most of these metabolites are necessary for defense systems in plants. Terpenes, quinone and tannins play an important role in odor and pigment formation and are used in antimicrobial research (Cowan, 1999; Silva and Fernandes, 2010). Cowan (1999) collected in 5 groups antimicrobial phytochemicals. Karou et al. (2007) reported that phenols constitute the largest group of herbal antimicrobial agents. In addition, antimicrobial activity of polyphenols and tannins in the plants were known for many years (Taguri et al., 2004).

The above-ground hexane extract of this subspecies did not demonstrate any antimicrobial activity on *S. aureus*, *M. luteus*, *P. aeruginosa* and *C. albicans*, while the above-ground hexane extract had moderate antimicrobial activity only on *E. coli*. The above-

ground chloroform, ethyl acetate and ethanol extracts showed no antimicrobial activity on the bacteria, whereas these extracts showed intermediate antifungal activity on yeast. The above-ground ethanol+aqueous and aqueous extracts were found to have moderate activity on *P. aeruginosa* and *M. luteus* and *C. albicans,* respectively. However, the activity on other microorganisms of the above-ground ethanol+aqueous and aqueous extracts could not be determined (Table 1). All below-ground extracts of the subspecies were observed to show intermediate antifungal activity on *C. albicans*. The below-ground ethyl acetate extract had moderate antimicrobial activity on only *S. aureus* from bacteria. Although below-ground ethanol extract showed moderate activity on *S. aureus*, the below-ground ethanol extract showed stronger antimicrobial activity on *M. luteus* and *P. aeruginosa*. The below-ground ethanol extract of this subspecies did not have any antimicrobial activity on only *E. coli* bacterium (Table 1). The below-ground ethanol extract of the subspecies can be used in the treatment of *S. aureus, M. luteus* and *P. aeruginosa* borne diseases.

Table 1. MIC values on different microorganisms of above and below-ground extracts (µg ml-1)
Çizelge 1. Topraküstü ve toprakaltı ekstrakların farklı mikroorganizmalar üzerindeki MİK değerleri (µg ml-1)

Plant Extracts *(Bitki Ekstrakları)*	*Staphylococcus aureus* ATCC 25923	*Micrococcus luteus* NRLLB1018	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Candida albicans* ATCC 10231
Above-ground hexane	3000	3000	1500	3000	3000
Below-ground hexane	>5000	3000	>5000	>5000	1500
Above-ground chloroform	3000	3000	3000	3000	1500
Below-ground chloroform	3000	3000	3000	3000	1500
Above-ground ethyl acetate	3000	3000	3000	3000	1500
Below-ground ethyl acetate	1500	3000	3000	3000	1500
Above-ground ethanol	3000	3000	>5000	3000	1500
Below-ground ethanol	1500	750	>5000	750	1500
Above-ground ethanol+aqueous	>5000	3000	3000	1500	>5000
Below-ground ethanol+aqueous	3000	3000	>5000	3000	1500
Above-ground aqueous	>5000	1500	3000	3000	1500
Below-ground aqueous	3000	3000	3000	3000	1500
DMSO	>5000	>5000	>5000	>5000	>5000

Similar results have been reported in antimicrobial studies of other *Heliotropium* species. Namely, the chloroform, petroleum ether and ethanol extracts of *H. subulatum* Hochst. ex DC. were applied to some bacteria (*Escherichia coli, Streptococcus pneumoniae, Bacillus subtilis, B. anthracis, Staphylococcus aureus*) and fungi (*Aspergillus fumigatus, A. niger, Rhizoctonia phaseoli* and *Penicillum chrysogenum*). The chloroform extract was found to show greater activity against *E. coli*, whereas petroleum ether extract was found to have better effect against *P. chrysogenum* (Singh et al., 2002). Some sterol and triterpenoids isolated from *H. ellipticum* Ledeb. were applied to bacteria and fungi and some of them showed to have the highest level of antimicrobial activity (Jain et al., 2001).

The hexane extract of *H. marifolium* Koen. ex Retz. were tested on the pathogenic bacteria (*E. coli, S. aureus*) and fungi (*A. niger ve P. chrysogenum*). The antimicrobial results were showed to possess high activity against *S. aureus P. chrysogenum E. coli* and *A. niger* (Singh and Dubey, 2001). Moreover, Radha et al. (2003) reported that methanol, ethyl acetate, chloroform and aqueous extracts of *H. marifolium* had antimicrobial activity.

In this study, the above-ground hexane extract had

antimicrobial activity on *E. coli*, while the below-ground hexane extract had antimicrobial activity on *C. albicans*. The above and below-ground ethyl acetate, chloroform, aqueous and ethanol extracts of subsp. *erzurumicum* were seen to have moderate antimicrobial activity on selected yeast (*C. albicans*). The below-ground ethyl acetate and ethanol extracts of subsp. *erzurumicum* exhibited antimicrobial activity on selected Gram-positive bacterium (*S. aureus*). Also, the below-ground ethanol extract of investigated subspecies possess strong antimicrobial activity on the selected Gram positive and negative bacteria, namely *M. luteus* and *P. aeruginosa*. In summary, in this study antimicrobial data are in harmony with Radha et al. (2003) and Sing and Dubey (2001) antimicrobial data.

Although secondary metabolites isolated from *H. filifolium* (Miers) Reiche showed significant antimicrobial activity on the Gram-positive bacteria, these metabolites showed inactive effect on the Gram-negative bacteria (Urzua et al., 2008). The below-ground ethanol extract of subsp. *erzurumicum* had strong antimicrobial activity on both Gram positive and Gram-negative bacteria, while the below-ground ethyl acetate extract had strong antimicrobial activity on only Gram-positive bacteria. On the other hand, the above-ground hexane and ethanol+aqueous extracts of subsp. *erzurumicum* possess moderate antimicrobial activity on only Gram-negative bacteria. We think that this case may be due to different cell wall structures of Gram positive and negative bacteria**.** Additionaly, the reason for this difference can be attributed to the fact that *H. filifolium* and subsp. *erzurumicum* have different secondary metabolities. Different metabolites have different antimicrobial activites such as cell wall complex, DNA interaction, enzyme inactivation, substrate loss, metal ion complex, binding to proteins, membrane destruction (Cowan, 1999).

Alcohol extract of *H. indicum* were applied to four Gram positive and Gram-negative bacteria, three fungi and two yeasts. Then, Rao et al. (2006) reported to show promising antimicrobial activity on selected all bacteria, fungi and yeasts. Methanol extract of *H. indicum* was tested against *P. aeruginosa, Shigella boydii, S. dysenteriae, S. paratyphi*, *Vibrio mimicus, E. coli, S. aureus, S. lutea, B. subtilis, B. megaterium, B.* cereus and *A. niger*. The strongest antimicrobial activity was reported to have on *P. aeruginosa*, moderate antimicrobial activity was on *S. lutea, B. subtilis, B. megaterium* and mild antimicrobial activity was on other bacteria (Yasmin, 2014). In this study, the strongest and moderate antimicrobial activity on both Gram negative and positive bacteria were found in below-ground ethanol extracts. Yasmin (2014) and Rao et al. (2006) antimicrobial data almost support in this study antimicrobial data. This is thought to be due to the use of similar organic solvents.

Jain and Sharma (1987) put forward to have positive antimicrobial activity on the pathogenic bacteria and fungi of some pyrrolizidine alkaloids isolated from *H. ellipticum*. Methanol and dichloromethane extracts of *H. dasycarpum* L. were tested on some bacteria (*E. coli, B. subtilis, Shigella flexinari*, *S. aureus, P. aeruginosa* and *S. typii*) and fungus (*C. albicans, A. flavus, Fusarium solani, C. glabrata* and *Microsporum canis*) and these extracts were found to have inactive antibacterial activity on the bacteria. The methanol extracts of *H. dasycarpum* showed a low antifungal activity (25 %) on only *Microsporum canis* (Ghaffari et al., 2013). The antifungal results of *H. dasycarpum* L. are in agreement with the antifungal results obtained from other species of the Boraginaceae family. Namely, methanol extracts of *Onosma griffiithii* showed 55% antifungal activity on *A. flavus* and 40% on *F. solani*, while n-butanol and ethyl acetate extracts did not show antifungal activity on *A. flavus* and *F. solani* (Ahmad et al., 2009). In this study, the above-ground chloroform, ethyl acetate and ethanol extracts and all below-ground extracts of this subspecies possesses moderate antifungal avtivity on *C. albicans*. The reason why Ghaffari et al. (2015), Ahmad et al. (2009) and in this study results are different is due to the different Boraginaceae taxa, the use of different types of organic solvents and fungi in the studies.

In another research, the methanol, n-hexane and ethylacetate extracts of *H. bacciferum* Forssk. were found to have an excellent antimicrobial activity on *E. coli, S. typhi, S. aureus, P. aeroginosa, E. carotovora, K. pneumoniae, B. atrophaeus* and *B. subtilis* (Ahmad et al., 2015). However, n-butanol and aqueous extracts showed inactive activity on *S. aureus* and *B. subtilis*, respectively. The above-mentioned extracts of *H. bacciferum* were applied to *C. albicans, Fusarium solani, A. niger, A. flavus*, *Trichoderma longibrachiantum* and significant antifungal results were obtained. According to the above findings, Ahmad et al. (2015) reported that *H. bacciferum* would be important in the treatment of various diseases. In this study antimicrobial findings are close to those of Ahmad et al. (2015). This may result from the application of similar organic solvents because similar organic solvents reveal similar secondary metabolites in plants.

When DPPH results of above and below-ground extracts of subsp. *erzurumicum* examined, all above and below-ground extracts were seen to have high antioxidant activity (Figures 1 and 2, Table 2). Especially above ground ethanol+aqueous, chloroform and below-ground ethanol extracts demonstrated the highest antioxidant activity compared to other plant extracts. For this, above ground ethanol+aqueous, chloroform and below-ground ethanol extracts of subsp. *erzurumicum* may be preferred as natural antioxidant sources in the future.

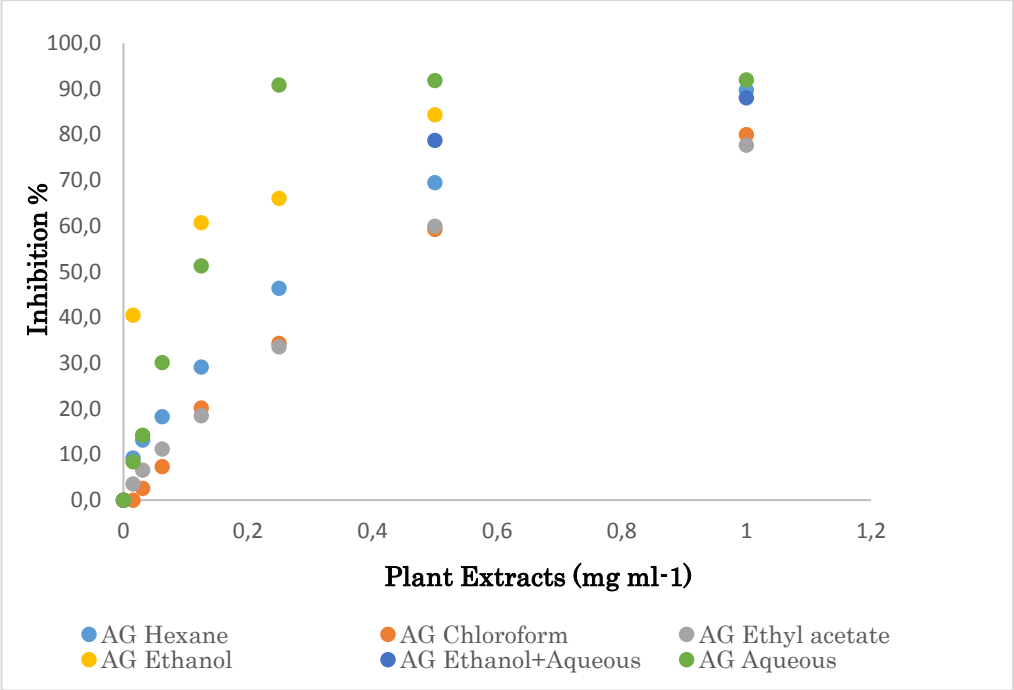

Figure 1. DPPH radical trapping activity of above-ground extracts AG: Above-Ground
Şekil 1. Topraküstü ekstraklarının DPPH radikalini yakalama aktivitesi AG: Topraküstü

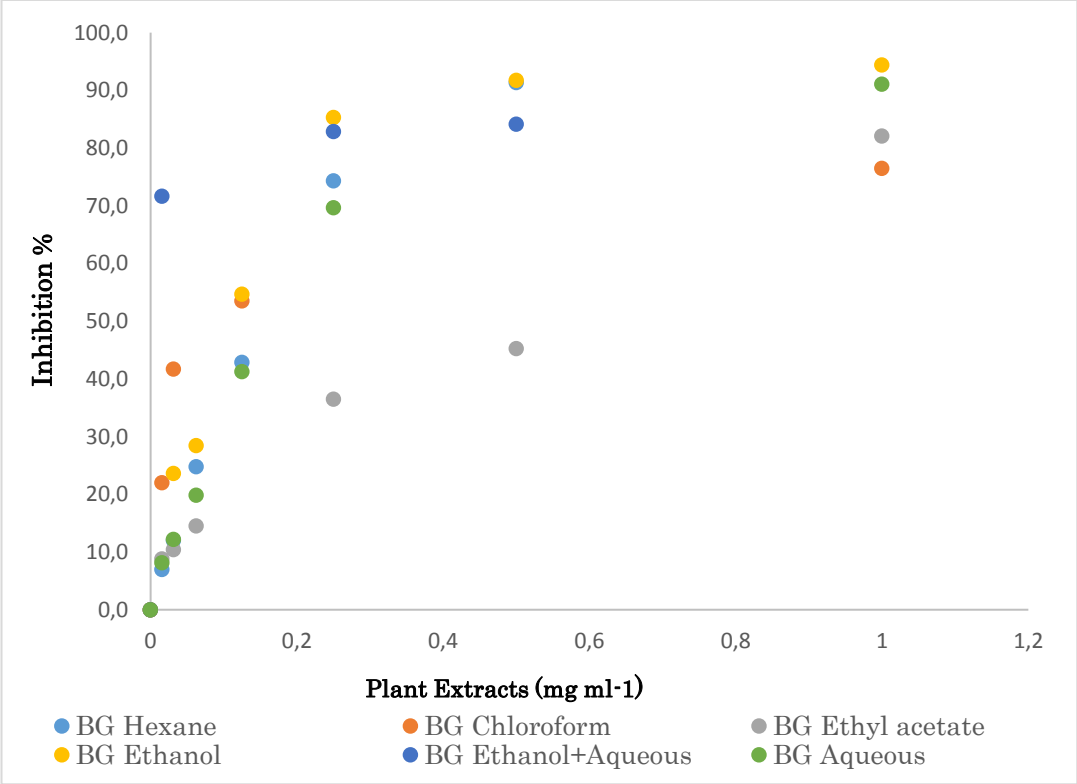

Figure2. DPPH radical trapping activity of below-ground extracts BG: Below-Ground
Şekil 2. Toprakaltı ekstraklarının DPPH radikalini yakalama aktivitesi BG: Toprakaltı

Antioxidant properties of petroleum ether, chloroform, aqueous and ethanol extracts of *H. indicum* were investigated according to DPPH and H_2O_2 methods and it was found that ethanol extracts had antioxidant properties (Sathosha et al., 2015). These researchers reported that tannin and flavonoids in *H. indicum* extracts are free radical scavengers. It has also been suggested that *H. indicum* extract will be a natural

oxidant source for the prevention of diseases including ageing due to various oxidative stress. On the other hand, Pragada et al. (2012) showed to have a good antioxidant activity of different extracts of *H. indicum*. Flovonids, phenolic compounds and resin isolated from *H. sinuatum* Miers., *H. sclerocarpum* Phil. were determined to have antioxidant properties, respectively (Modak et al., 2005, 2009; Goyal and Sharma, 2014). The dichloromethane and methanol, chloroform extracts of *H. glutinosum* Phil., *H. taltalense* Phil. and *H. zeylanicum* (Burm.) Lam. were seen to have antioxidant activity, respectively (Modak et al., 2007, 2009; Goyal and Sharma, 2014).

Table 2. IC_{50} values of above and below-ground extracts

Çizelge 2. Topraküstü ve toprakaltı ekstrakların IC_{50} değerleri

Plant Extracts (*Bitki Ekstrakları)*	IC_{50} (mg ml-1)
BHA	0.019
Above-ground hexane	0.133
Below-ground hexane	0.289
Above-ground chloroform	0.097
Below-ground chloroform	0.407
Above-ground ethyl acetate	0.564
Below-ground ethyl acetate	0.406
Above-ground ethanol	0.114
Below-ground ethanol	0.067
Above-ground ethanol+aqueous	0.011
Below-ground ethanol+aqueous	0.318
Above-ground aqueous	0.163
Below-ground aqueous	0.121

Antioxidant activity was seen in ethyl acetate, n-hexane and aqueous extracts of *H. strigosum* (Hussain et al., 2010). Also, the dichloromethane extracts of *H. subulatum* displayed significant antioxidant activity (Singh et al., 2017). Moreover, the flower, leaf, stem and root extracts of *H. bacciferum* exhibited noteworthy antioxidant activity (Al-Snafi, 2018). The above-mentioned antioxidant findings are consistent with in this study antioxidant findings. According to total phenolic content results, phenolic contents of both below and above-ground chloroform and below-ground ethanol+aqueous extracts of subsp. *erzurumicum* were the highest (Table 3). We think that the above and below-ground chloroform, below-ground ethanol+aqueous extracts of this plant may be used as antioxidant source.

The DNA interaction results are presented in Figures 3-5. 1 and 2 lanes in Figures 3-5 belong to pBR322 DNA+H_2O and pBR322 DNA+DMSO control groups, respectively. According to data in Figure 3, the below-ground aqueous, (Lane 3), ethyl acetate (Lane 4), ethanol (Lane 5) and above-ground aqueous (Lane 6) extracts have enhancing effect in the concentration of open ring form of pBR322 plasmid DNA. However, it was determined that below-ground aqueous extract was more effective than others in the formation of open ring form of pBR322 plasmid DNA. The above-ground ethanol (Lane 3), ethyl acetate (Lane 4), ethanol+aqueous (Lane 5) and below-ground ethanol+aqueous (Lane 6) and hexane (Lane7) extracts have no effect on pBR322 plasmid DNA (Figure 4). When the results of Figure 5 examined, the extracts of below and above-ground chloroform (Lanes 3 and 5) and above-ground hexane (Lane 6) have increasing effect in the concentration of open ring form of pBR322 plasmid DNA.

Table 3. Phenolic contents of above and below-ground extracts

Çizelge 3. Topraküstü ve toprakaltı ekstrakların fenolik içerikleri

Plant Extracts *(Bitki Ekstrakları)*	Phenolic contents *(Fenolik bileşikler)* (µg GAE ml-1)
Above-ground hexane	*
Below-ground hexane	*
Above-ground chloroform	241.18
Below-ground chloroform	169.28
Above-ground ethyl acetate	*
Below-ground ethyl acetate	*
Above-ground ethanol	84.31
Below-ground ethanol	84.31
Above-ground ethanol+aqueous	*
Below-ground ethanol+aqueous	167.10
Above-ground aqueous	*
Below-ground aqueous	10.24

*: incalculated total phenolic contents

It has been observed that below-ground chloroform and aqueous extracts are more effective than other extracts on the formation of the open ring form of pBR322 plasmid DNA.

The above and below-ground ethanol extracts of *Centranthus longiflorus* subsp. *longiflorus* were determined to show significant effect on pBR322 plasmid DNA (Ayar and Kandemir, 2020). In a similar study with *Leucojum aestivum* L., below-ground ethanol extracts of *L. aestivum* displayed to have highly effect on pBR322 plasmid DNA (Hundur et al., 2018). However, the above and below-ground ethanol, ethyl acetate and dichloromethane extracts of *Linaria corifolia* Desf., were determined to have protective activity on pBR322 plasmid DNA (Gul et al., 2017).

CONCLUSION

Consequently, the below-ground extracts of this subspecies had more effective antioxidant and biological activity than the above-ground extracts. Therefore, it shows that extracts made with different solvents of this subspecies can be used as a source in

the pharmaceutical industry and traditional medicine. Due to the above-mentioned features, we believe that the studied subspecies can take place in Turkey's medicinal plants. *H. samolifolium* subsp*. erzurumicum* is one of the rare endemic taxa for Flora of Turkey. Both the protection of this taxon and required sensitivity must be given the necessary importance to use it in the most efficient way.

Figure 3. Agarose gel electrophoresis diagram based on the interaction of pBR322 plasmid DNA.
Şekil 3. pBR322 plazmit DNA'nın etkileşimine dayalı agaroz jel elektroforez diyagramı
Lane 1:pBR322 DNA+H_2O control; Lane 2:pBR322 DNA+DMSO control; Lane 3:pBR322 DNA+BG aqueous extract; Lane 4: pBR322 DNA+BG ethyl acetate extract; Lane 5: pBR322 DNA+BG ethanol extract; Lane 6: pBR322 DNA+AG aqueous extract.

Figure 4. Agarose gel electrophoresis diagram based on the interaction of pBR322 plasmid DNA.
Şekil 4. pBR322 plazmit DNA'nın etkileşimine dayalı agaroz jel elektroforez diyagramı
Lane 1:pBR322 DNA+H_2O control; Lane 2:pBR322 DNA+DMSO control; Lane 3:pBR322 DNA+AG ethanol extract; Lane 4: pBR322 DNA+AG ethyl acetate extract; Lane 5: pBR322 DNA+AG ethanol+aqueous extract; Lane 6: pBR322 DNA+BG ethanol+aqueous extract; Lane 7: pBR322 DNA+BG hexane extract.

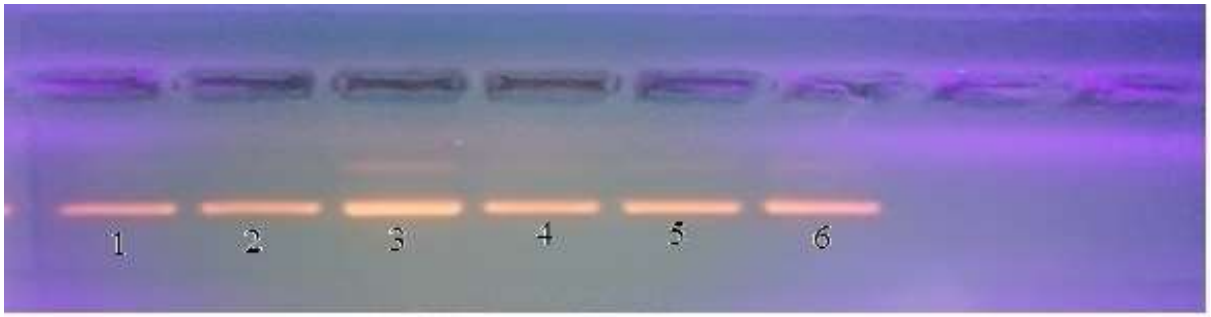

Figure 5. Agarose gel electrophoresis diagram based on the interaction of pBR322 plasmid DNA.
Şekil 5. pBR322 plazmit DNA'nın etkileşimine dayalı agaroz jel elektroforez diyagramı
Lane 1:pBR322 DNA+H_2O control; Lane 2:pBR322 DNA+DMSO control; Lane 3:pBR322 DNA+BG chloroform extract; Lane 4: pBR322 DNA+BG hexane extract; Lane 5: pBR322 DNA+AG chloroform extract; Lane 6: pBR322 DNA+AG hexane extract.

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Statement of Conflict of Interest

Author has declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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