KSÜ Tarım ve Doğa Derg 25 (Ek Sayı 2): 316-325, 2022
KSU J. Agric Nat 25 (Suppl 2): 316-325, 2022
https://doi.org/10.18016/ksutarimdoga.vi.1062822

Evaluation of Toxic Effects of *Dictamnus albus* L. Extracts on PC-12 and SHSY-5Y Cell Lines and Investigation of Antioxidant Activity

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ABSTRACT

This study investigated the antioxidant and cytotoxic properties of the *Dictamnus albus* L. plant grown in Türkiye. The aerial parts and roots of the plant were evaluated qualitatively in terms of chemical content. Total phenol and flavonoid amounts were calculated by spectrophotometric methods, antioxidant activity was tested with DPPH and ABTS radical scavenging activity assay. In addition, cell viability determination in PC-12 and SHSY-5Y cell lines was evaluated by performing MTT (3-4,5-dimethyl-thiazolyl-2,5-diphenyltetrazolium bromide) test. According to the results, both parts of the plant gave negative results in the tannin, cyanogenetic glycoside, anthraquinone, cardiac glycoside, and anthocyanoside identification tests, while they gave positive results in the alkaloid, coumarin, saponin, carbohydrate identification tests. While the total amount of phenol was calculated as 77.13 ± 5.73 mg_{GAE} $g_{exracts}$-1 in the extract prepared with methanol from the aerial parts of the plant (D.A Herba), it was calculated as 43.81 ± 9.49 mg_{GAE} $g_{exracts}$-1 in the extract prepared from the roots (D.A Root). The total flavonoid content could only be calculated in the D.A Herba extract (19.11 ± 0.16 mg_{CA} $g_{extract}$-1). Although the DPPH radical scavenging effect of the extracts was higher in D.A Herba extract, the ABTS radical scavenging effects were found similar in both D.A Herba and D.A Root extracts. According to the toxicity test, D.A Root extract reduced the viability below 50% (43.17 ± 3.44%) at 500 µg mL-1, but D.A Herba extract was found to be more toxic at the same concentration with 19.53 ± 0.183% in the PC-12 cell line. However, D.A. Herba and D.A.Root extract increased cell proliferation in the SHSY-5Y cell line at 3.25 µg/mL concentrations with 122.87±6.29 and 112.78±7.00%, respectively. The results suggest that *D. albus* may be a promising candidate for the new phytopharmaceuticals due to its neuroprotective effects.

Biology

Research Article

Article History
Received : 25.01.2022
Accepted : 30.05.2022

Keywords
Antioxidant
PC-12
Rutaceae
SHSY-5Y

Dictamnus albus L' a Ait. Ekstrelerin PC-12 ve SHSY-5Y Hücre Hatlarında Toksik Etkilerinin Değerlendirilmesi ve Antioksidan Aktivitesinin Araştırılması

ÖZET

Bu çalışmada, Türkiye'de yetişen *Dictamnus albus* L. bitkisinin, antioksidan ve sitotoksik özellikleri araştırılmıştır. Bitkinin topraküstü ve kök kısımları kimyasal içerik bakımından kalitatif olarak değerlendirilmiştir. Toplam fenol ve flavonoit miktarı spektrofotometrik yöntemlerle hesaplanmış, antioksidan aktivitesi DPPH ve ABTS radikalini süpürücü aktivite deneyi ile test edilmiştir. Ayrıca PC-12 ve SHSY-5Y hücre hatlarında canlılık tayini MTT (3-4,5-dimetil-tiyazolil-2,5-difeniltetrazolyum bromür) testi yapılarak değerlendirilmiştir. Sonuçlara göre; bitkinin her iki kısmı da tanen, siyanogenetik glikozit, antrakinon, kardiyak glikozit ve antosiyanozit teşhis deneylerinde negatif sonuç verirken, alkaloit, kumarin, saponin, karbonhidrat teşhis deneylerinde pozitif sonuç vermiştir. Bitkinin topraküstü kısımlarından metanol ile hazırlanan

Biyoloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 25.01.2022
Kabul Tarihi : 30.06.2022

Anahtar Kelimeler
Antioksidan
PC-12
Rutaceae
SHSY-5Y

ekstresinde (D.A Herba) toplam fenol miktarı 77,13 ± 5,73 mg_{GAE} g_{ekstre}-1 olarak hesaplanırken, köklerinden hazırlanan ekstresinde (D. A Root) ise 43,81 ± 9,49 mg_{GAE} g_{ekstre}-1 olarak hesaplanmıştır. Toplam flavonoit içeriği ise yalnızca D.A Herba ekstresinde hesaplanabilmiştir (19,11 ± 0,16 mg_{CA} g_{ekstre}-1). Ekstrelerin DPPH radikalini süpürücü etksi D.A Herba ekstresinde daha yüksek bulunurken, ABTS radikalini süpürücü etkileri her iki ekstrede de benzer bulunmuştur. Toksisite deneyi sonuçlarına göre, PC-12 hücre hattında 500 µg mL-1'de D.A Root ekstresi canlılığı % 50'nin altına düşürmüş (% 43,17 ± 3,44), ancak D.A Herba ekstresi aynı konsantrasyonda %. 19,53 ± 0,183 ile daha toksik etkili bulunmuştur. Ancak, D.A. Herba ve D.A.Root ekstresi SHSY-5Y hücre hattında 3,25 µg mL-1 konsantrasyonlarda sırasıyla % 122,87 ± 6,29 ve 112,78 ± 7,00 canlılık oranı ile hücre proliferasyonu arttırmıştır. Sonuçlar *D. albus* bitkisinin nöroprotektif etkileri sebebiyle yeni fitofarmasötiklerin için potansiyel adaylar olabileceğini düşündürmektedir.

To Cite : İlgün S, Karatoprak Şeker G 2022 Evaluation of Toxic Effects of *Dictamnus albus* L. Extracts on PC-12 and SHSY-5Y Cell Lines and Investigation of Antioxidant Activity. KSU J. Agric Nat 25 (Ek Sayı 2): 316-325. https://doi.org/10.18016/ksutarimdoga.vi.1062822

Atıf Şekli: İlgün S, Karatoprak Şeker G 2022. *Dictamnus albus* L' a Ait. Ekstrelerin PC-12 ve SHSY-5Y Hücre Hatlarında Toksik Etkilerinin Değerlendirilmesi ve Antioksidan Aktivitesinin Araştırılması . KSÜ Tarım ve Doğa Derg 25 (Suppl 2): 316-325. https://doi.org/10.18016/ksutarimdoga.vi. 1062822

INTRODUCTION

Dictamnus albus L. is known as "Purple Dittany" or "Burning Bush", are shrub-shaped plants that have been grown as ornamental plants for centuries. The plant is belonging to the Rutaceae family and has a woody stem at the base, pinnate leaves, and large purplish-pink and white zygomorphic flowers. The whole plant is covered with aromatic glandular hairs with flammable essential oils (Compton and Akeroyd, 2019). *D. albus*, has a natural and wide distribution area almost all over the world throughout South and Central Europe, temperate Asia, and the Himalayas (Nissar et al., 2021). *D. albus*, which is accepted as a polymorphic species due to its distribution in a wide geographical area, has been tried to be distinguished by taxonomists (Compton and Akeroyd, 2019). Many researchers have accepted this plant as the only species in the world. All *Dictamnus* species, which are called different species in the literature, have been accepted as synonyms of *Dictamnus albus* (Plantlist 2021).

Dictamnus albus, which is generally known as an attractive decorative plant, has a wide variety of therapeutic properties due to its bioactive components (Gnatiuk 2019). The investigations contain various information that the plant is used in the cure of diseases by people in different countries traditionally. In Türkiye; flowering branches of *D. albus* are used as stomachic, stimulant, tonic and antipyretic and local names of the plant are "Akgirit otu or Gazel otu" (Baytop 2009). The plant, known under two different names as *Dictamnus dasycarpus* Turcz and *Dictamnus angustifolius* G. Don ex Sweet. in traditional Chinese medicine and "Cortex Dictamni" has been used in rheumatism, bleeding, itching, jaundice, chronic hepatitis, and skin diseases for centuries (LV et al., 2015). Indians have traditionally used the plant for its emmenagogue and abortive effects. It has been used in Israel to treat hypertension and ocular ailments such as cataracts, conjunctivitis, and diabetic retinopathy. In Bulgaria and Korea, people use this plant as a sedative, antispasmodic, diuretic, and it is stated to have anti-cancer effects. It is reported to be utilized as a tea mixture in the treatment of mental disorders such as neurasthenia, hysteria, and schizophrenia in Serbia. In addition, it is known that especially the root bark of the plant is used in the treatment of leprosy, cough, and amenorrhea (Nissar et al., 2021).

The main chemical components of *Dictamnus* species were determined as limonoids, alkaloids, sesquiterpenes, flavonoids, coumarins, terpenoids, and steroidals. Among these compounds, especially limonoids and quinoline alkaloids were identified as specific components of *Dictamnus* species. The biological activities of these active constituents are quite diverse. Limonoids and quinoline alkaloids are significant compounds with anticancer, anti-inflammatory, antimicrobial, antioxidant, antiviral, immunosuppressing, neuroprotective, and vascular-relaxing properties (Gao et al.,2021).

This study carried out various bioactivity tests of extracts prepared from roots and aerial parts of *D. albus* grown in Türkiye. The total phenol and total flavonoid amount of the extracts were analyzed by spectrophotometric methods. The toxicity profile of the plant was evaluated on PC-12 and SHSY-5Y cell lines and their antioxidant activities were tested

using radical scavenging methods.

Fundamentally, this research aims to evaluate the neuroprotective effects of the extracts of the *Dictamnus albus* plant grown in Türkiye, used for its therapeutic properties in different areas of the world, and to measure the antioxidant activity. In addition, different parts of the plant (root and herba), which traditionally used root bark in the treatment, were evaluated in terms of bioactivity for the first time by us. The obtained data are important in terms of comparing the secondary metabolites contained in the same species studied from different regions of the world and the resulting bioactivity tests and guiding the studies. Our preliminary research on this plant, which is accepted as a single species in the whole world and spreads in Türkiye, contains promising results.

MATERIAL and METHODS

Plant Material

Dictamnus albus was collected from Mecidiye/Keşan area from Edirne. The collected specimens were identified by Dr. Necmettin Güler and plant material is being stored in Ankara University Pharmacy Herbarium under voucher number AEF 30894. The roots and aerial parts of the plant were fragmented into two parts and dried under shade at room temperature.

Extraction of Plant Materials

After the root and aerial parts of the plant were roughly pulverized, they were placed in a shaker water bath to prepare methanol extracts. Plant materials to which methanol was added for 3 days were filtered at the end of each day and the filtrates were combined. Then the solvents were removed in vacuo and the extracts were obtained. All the extracts were lyophilized as powder and stored at -20°C until used in experiments.

Total Phenol Content

The total phenol content of the extracts was measured by revising the methods of Re et. al (1999). The samples were prepared by mixing extracts (50 µL), distilled water (3.95 mL), Folin Ciocalteu reagent (250 µL), and 20% Na_2CO_3 (750 µL), and kept in a water bath at 25 °C for 2 hours. The samples' absorbance was finally read at 760 nm and gallic acid was used as the reference to calculate the total phenol content (Singleton et al.,1999).

Total Flavonoid Content

The total flavonoid content of the extracts was measured by revising the methods of Singleton et al. with the colorimetric aluminum chloride assay. 4 mL distilled water was added to the 1 mL extract. A 0.3 mL solution of $NaNO_2$ (5%) was added to the mixture. After 5 minutes, 0.3 mL of a 10% $AlCl_36H_2O$ solution was added. Then 2 mL of 1 M NaOH was added, and the total volume was made up to 10 mL with distilled water. The test sample's absorbance was read at 510 nm. Catechin was used as the reference standard to calculate the total flavonoid content (Zhishen et al.,1999).

Phytochemical Analysis

Qualitative analyzes of the plant prepared from the roots and aerial parts were made according to the standard specified methods (Tanker and Sakar 1991). The presence of alkaloids, flavonoids, carbohydrates, coumarins; tannins, saponins, cyanogenetic glycosides, cardiac glycoside, anthraquinone, and anthocyanoside in the root and aerial parts of *D. albus* were evaluated.

Antioxidant Activity Assays

The antioxidant activity of the *D. albus* extracts was determined using DPPH radical scavenging assay (Gyamfi et al. 1999). In a test tube, the extract was mixed with 450 mL of Tris-HCl buffer and 1.0 mL of 0.1 mM DPPH after being dissolved in the proper quantity of MeOH. The extracts were measured at 517 nm after 30 minutes of incubation at room temperature in the dark. Butylated hydroxytoluene (BHT) was used as a positive control.

The antioxidant activities of the extracts were also measured by another method, $ABTS^{+\bullet}$ radical scavenging activity. 7 mM ammonium ABTS salt was dissolved in water and treated with 2.45 mM potassium persulfate. A dark blue solution was obtained by keeping this mixture at room temperature for 12-16 hours. The solution was then diluted with ethanol to have an absorbance of 0.7 at 734 nm. To measure the reaction kinetics, radical solution (990 µL) was added to the extract (10 µL) and absorbance was taken at 734 nm for 30 minutes at 1-minute intervals. Results were expressed in terms of antioxidant capacity equivalent to Trolox (Re et al., 1999).

Cell Viability Assay

PC-12 (ATCC, CRL-1721™ Passage no:8) and SHSY-5Y (ATCC, CRL-2266™ Passage no:5) were purchased from American Type Culture Collection (Manassas, VA, USA). Cells were seeded into a 75 cm2 flask using Roswell Park Memorial Institute Medium (RPMI-1640 30-2001™) and Dulbecco's Modified Eagle's Medium (DMEM 30-2002™) with 10% fetal bovine serum (FBS 30-2020™) and 1% penicillin-streptomycin (Gibco-Invitrogen, Grand Island, NY, USA) respectively. Then cells were grown in the incubator (at 37°C in an atmosphere supplemented with 5% CO_2) to reach the appropriate

density.

A total of $1x10^5$ cells per well were seeded in 96-well plates. Extracts were prepared by dissolving in the medium containing 1% dimethyl sulfoxide (DMSO) at a 4 mg mL^{-1} concentration. Cells were treated with varying doses of extract (7.8; 15.6; 31.25; 62.5; 125; 250; 500; 1000; and 2000 µg/mL) after 24 hours of incubation and allowed to re-incubate for another 24 hours. The medium was then removed and MTT (5 mg mL-1 of stock in PBS) was added. Cells were incubated with MTT dye for an additional 2 hours. At the end of the incubation period, absorbances (solution in each well) were measured at 570 nm in a microplate reader.

Statistical analysis

All statistical analyzes were made with the SPSS 12 (SPSS for Windows, 12.0, SPSS Inc. Chicago, IL, USA) statistical program. Analysis of variances was applied according to the ANOVA procedure. According to the results of Levene statistics applied to test the homogeneity of variance, Dunnet T3 test was used in the experiments where the assumption was not provided for the variable($p<0,05$). In the experiments where the assumption was provided, the analysis was made with the Tukey test($p>0,05$). IC50 values were calculated using a nonlinear regression algorithm.

RESULTS AND DISCUSSION

D. albus, which has a wide distribution area and is represented by a single species all over the world, has various biological activities related to its natural compounds. The plant is used especially in the treatment of various diseases in Chinese Folk Medicine. Cortex Dictamni has been used for the treatment of rheumatism, bleeding, itching, jaundice, chronic hepatitis, and skin diseases in China (Lv et al., 2015). The plant contains primarily limonoids, furoquinoline alkaloids, flavonoids, coumarins, sesquiterpene, and sesquiterpene glycosides (Chang et al., 2002). Due to these bioactive compounds, the plant has valuable medicinal properties such as anti-inflammatory, anti-tumor, antibacterial, and immune-regulating functions (Cao et al., 2022).

In the present study, qualitative analyzes were carried out to have preliminary information about the secondary metabolites of the root and aerial parts of *D. albus* species grown in Türkiye. The presence of alkaloids, flavonoids, carbohydrates, coumarins, tannins, saponins, cyanogenetic glycosides; cardiac glycoside; anthraquinone, and anthocyanoside in the parts of plants was tested qualitatively. These analyzes are based on visual observation of color change or precipitate formation after the addition of certain reagents. Phytochemical analysis results are given in Table 1. According to the results, the same results were obtained according to the diagnostic reactions performed on both the aerial parts and root parts of the plant (except for the flavonoid detection reaction). The presence of alkaloids, coumarins, carbohydrates, and saponins was detected in the aerial parts and root of the plant, while tests for cyanogenetic glycosides, cardiac glycosides, anthraquinones, and anthocyanosides gave negative results. The flavonoid identification reaction gave positive results only in the aerial parts. This result is thought to be related to the inability to quantitatively calculate the total amount of flavonoids in root extracts (Table 2). In a study conducted in Indonesia, the phytochemical content of *D. albus* roots was evaluated in terms of alkaloid, flavonoid, carbohydrate, coumarin, saponin, tannin, triterpenoid, and steroids, and similar results were obtained with our analysis. However, while the presence of saponin in the roots of the plant was not detected in the study, the saponin identification reaction gave a positive result in our study (Rohim et al., 2018).

Table 1. Phytochemical analysis of *D. albus* aerial parts and roots
Çizelge 1. *D. albus* topraküstü kısımları ve köklerinin fitokimyasal analizi

Parts of Plant	Alk*	Flav*	Carb*	Coum*	Tan*	Sap*	Cyano gly*	Card. Gly*	Anth*	Antho*
Aerial parts	+	+	+	+	-	+	-	-	-	-
Root	+	-	+	+	-	+	-	-	-	-

*Alk= alkaloid; Fla= flavonoid; Carbo= Carbohydrate; Cou= coumarin; Tan= tannin; Sap= saponin; Cyano Gly=Cyanogenetic glycosides; Card. Gly=Cardiac glycoside; Anth=Anthraquinone; Antho=Anthocyanoside

The total phenol and flavonoid content of the plant was studied by preparing methanol extracts. Total phenol and flavonoid contents of the extracts were calculated as equivalent to gallic acid and catechin, respectively. The total phenol content of the plant was calculated as 77.13 ± 5.73 mg_{GAE} $g_{extract}$-1 in the D.A. Herba extract and 43.81 ± 9.49 mg_{GAE} g_{extrac}t-1 in the D.A. Root extract. However, the total flavonoid

content in D.A Herba was also higher than in D.A Root (Table 2). In a study in which the content analysis and antioxidant capacity of some plants belonging to the Rutaceae family were determined, the total flavonoid content of the ethanol and methanol extract prepared from the aerial parts of the *D. albus* species was examined. According to the results of this study, the total flavonoid content in the methanol extract of the aerial parts of the *D. albus* collected from Serbia (7.27±0.12 mgrutin g-1) was lower than the methanol extract of the aerial parts of the *D. albus* plant collected from Türkiye (19.11 ± 0.16 mg_{CA} $g_{extract}$-1) (Pavlović et al. 2018). It is known that changes in the physiological activities of the plant significantly affect its chemical content, depending on the environment in which it lives. In addition, different methods applied and different concentrations are also reflected in the results obtained.

Table 2. Total phenol and total flavonoid contents of *D. albus* extracts
Çizelge 2. *D. albus ekstrelerinin toplam fenol ve flavonoit içeriği*

Extracts	Total phenol [$mg_{GAE}/g_{extract}$]	Total flavonoid [$mg_{CA}/g_{extract}$]
D.A. Herba	77.13 ± 5.73	19.11 ± 0.16
D.A. Root	43.81 ± 9.49	n.d*

The data are presented as mean ± standard error (n=3), (*n.d= not determination)

Many studies have shown that plants have significant antioxidant activity due to the natural chemicals they contain (Kähkönen et al. 1999, Karatoprak et al. 2017). In addition, studies show that many different methods are used to determine the antioxidant capacity of secondary metabolites with different chemical properties in plant extracts (Alam et al. 2013, Yucel et al 2017, Borjan et al. 2020). Thus, by applying different methods, more detailed information about the antioxidant effects of plants can be obtained.

The antioxidant capabilities of *D. albus* extracts were measured using DPPH• and $ABTS^{•+}$ radical scavenging effects in this research. The extracts' radical scavenging effects were investigated at concentrations ranging from 0.5mg mL-1 to 4mg mL-1. According to the DPPH• radical scavenging method, it was determined that D.A Herba extract at 4mg mL-1 concentration showed higher activity (56.86 ± 2.4%) than D.A Root extract (34.56 ± 2.77%). Furthermore, the antioxidant capacity of the extracts was compared with BHT, one of the most known synthetic antioxidants. For BHT, the % inhibition was found to be 85.4 ± 3.02 at 4mg mL-1 concentration. (Table 3). In other words, although the extracts are not as effective as BHT, they show moderate antioxidant capacity In a recent study, the antioxidant capacity of the parts of the *D. dasycarpus* Turcz (root whisker, core stem, and leaf) other than the root bark, which is known to be used in treatment, were evaluated. According to the results obtained, the highest DPPH radical scavenging effect was detected in the leaves of the plant (IC50:.0.133±0.23 µg mL-1), while the weakest effect was calculated in the root extracts (IC50: 3.681±0.56 µg mL-1) (Cao et al., 2022). Similarly, in this study, root extracts were found to be less effective than herba extracts (Tables 3 and 4). In a study investigating the antioxidant capacity of the *D. albus* from Indonesia, the inhibition was found to be 82%, especially at 50µg mL-1 of the root extract and it was stated that it had a high antioxidant effect (Rohim et al., 2018). This data obtained by Rohim et. al. is quite different when compared to our study. This dissimilarity may be due to differences in the extraction methods and test procedures. In addition, since plants may show different physiological characteristics according to the regions where they grow, they may have different chemical contents. In another study, the IC50 values of methanol and ethanol extracts prepared from the aerial parts of the plant were calculated as 59.80 ± 1.53 µg mL-1 and 76.48 ± 2.30 µg mL-1, respectively, and it was recorded to be moderately effective compared to rutin (Pavlović et al. 2018).

Table 3. DPPH• radical scavenging activity of *D. albus* extracts
Çizelge 3. *D. albus ekstrelerinin DPPH● radikali süpürücü aktiviteleri*

	%Inhibition			
Samples	4 mg/mL	2 mg/mL	1 mg/mL	0.5 mg/mL
D.A. Herba	56.86 ± 2.4^{e}	40.89 ± 2.71^{d}	$29.56 \pm 2.12^{b,c}$	$24.99 \pm 3.18^{a,b}$
D.A. Root	$34.56 \pm 2.77^{c,d}$	$26.19 \pm 3.17^{a,b}$	$23.32 \pm 5.57^{a,b}$	19.25 ± 2.83^{a}
BHT	85.4 ± 3.02^{f}	82.3 ± 3.01^{f}	78.8 ± 3.8^{f}	66.0 ± 4.5^{g}

Another frequently used method to measure antioxidant activity is ABTS radical scavenging activity assay. According to the results obtained with this method, when the extracts were compared with the positive control BHT, D.A Herba extract and D.A Root extract showed similar antioxidant effects at 4 mg mL-1, but not as effective as BHT. However, it was determined that the D.A root extract showed very weak antioxidant activity compared to D. A Herba extract in the concentration range of 0.5mg mL-1 - 2 mg mL-1 (Table 4).

Table 4. ABTS•+radical scavenging activity of *D. albus* extracts
Çizelge 4. *D. albus ekstrelerinin ABTS•+radikali süpürücü aktiviteleri*

	TEAC* (mmol/L/Trolox)			
Samples	**4 mg/mL**	**2mg/mL**	**1mg/mL**	**0.5mg/mL**
D.A. Herba	2.53 ± 0.00^{a}	$2.52 \pm 0.01^{*}$	$2.19 \pm 0.19^{+}$	1.58 ± 0.13^{I}
D.A. Root	2.52 ± 0.07^{a}	$2.13 \pm 0.18^{**}$	$1.35 \pm 0.14^{++}$	0.064 ± 0.06^{II}
BHT	2.93 ± 0.2^{b}	$2.57 \pm 0.8^{***}$	$2.55 \pm 0.9^{+++}$	2.50 ± 0.1^{III}

Values presented as mean ± standard errors (n = 3), with statistical analyses performed using the Tukey comparison test. Same lower case letter (a–b), and the same symbols (*–***), (+–+++), and (I–III) are not significantly (p>0.05) different.

Values presented as mean ± standard errors (n = 3), with statistical analyses performed using the Tukey comparison test. The same lower case letter (a–g) is not significantly (p>0.05) different.

Neurotoxic effects of extracts prepared from *D. albus* species grown in Türkiye were investigated on PC-12 and SHSY-5Y cells. Studies show that SHSY-5Y and PC-12 cell lines are widely used in the investigation of neuronal cytotoxicity (Siddiqui et al. 2021; Sharma et al., 2021; Zhang et al 2022). SHSY-5Y cells are used in neuronal differentiation, metabolism, neurodegenerative and neuroadaptive mechanisms, neurotoxicity, and neuroprotective research. PC-12 cells have similar properties to neuroblasts and neurons. PC-12 cells are frequently used to investigate cellular events such as proliferation, differentiation, cell viability, and apoptosis, as well as mechanisms involving neuronal repair, neuroprotection, and neurotoxicity (Wang et al., 2019).

D. A. Herba was applied to SHSY-5Y and PC-12 cells at varying concentration ranges (3.25-1000 µg mL-1), and cell viability was observed. Following, it was determined that D. A. Herba was toxic to SHSY-5Y cells at high concentrations, while it increased the proliferation of cells at low concentrations. In SHSY-5Y cells, it was observed that the viability decreased below 50% in the concentration range of 250-1000 µg mL-1, while an increase in cell viability was observed in the concentration range of 3.25-31.25 µg mL-1 (Figure 1). D. A. Herba is seen to be highly toxic, especially at a concentration of 1000 µg mL-1 (8.116 ± 1.98%).

In the PC-12 cell line, it was determined that D.A Herba showed high toxicity in the concentration range of 250-1000 µg mL-1 and reduced the viability below 50%. No toxic effect of the extract was observed in the concentration range of 125 and 3.25 µg mL-1 (Figure 2). As a result, it was determined that D.A Herba at concentrations of 1000 and 500 µg/mL was significantly toxic to both PC-12 and SHSY-5Y cell lines.

To evaluate the toxic effect of D. A Root extract on SHSY-5Y and PC-12 cells, it was applied for 24 hours under the same conditions. According to the results obtained, D. A Root extract was found to have a toxic effect on the SHSY-5Y cell line in the concentration range of 125-1000 µg mL-1 and it was observed that it reduced the viability below 50%. At the lowest concentration (3.25 µg mL-1), the extract increased cell proliferation (112.784 ±7.07%) (Figure 3).

When the effects of D.A Root extract on the PC-12 cell line were evaluated, it was observed that it significantly inhibited cell viability at a concentration of 1000 µg mL-1 (11.504 ± 1.44). However, no significant toxic effect on cells was observed in the concentration range of 3.25-250 µg mL-1 (Figure 4). In particular, D.A root extract reduced the viability to below 50% (47.931 ± 0.79) at 125 µg mL-1 concentration in the SHSY-5Y cell line, while the viability was 76.260±0.68% at the same concentration in the PC 12 cell line.

As a result, when the toxicity of the extracts on PC-12 and SHSY-5Y cells is compared in terms of IC50 values, it can be observed that D. A. Herba extract has less toxic effects on both cell lines, especially at decreasing concentrations (Table 5).

Many studies have been carried out to investigate the neuroprotective effects of this plant in the literature. (Jeong et al., 2010; Choi et al., 2011; Sun et al., 2015; Yoon et al., 2010). However, the toxicity of the extracts of *D. albus* grown in Türkiye on PC-12 and SHSY-5Y cells was investigated by us for the first time. Therewithal, studies have generally focused on limonoids isolated from the plant and identified as major active components. Sun et al. (2015) determined that six limonoids (dictangustones A-F) isolated from the root bark of *D. angustifolius* (Sym:

D. albus) were nontoxic to SH-SY5Y cells and showed considerable neuroprotective effect against oxidative stress-induced neuronal death. Furthermore, limonoids extracted from the plant have been shown to exhibit considerable neuroprotective action in rat cortical cells against glutamate-induced neurotoxicity (Yoon et al., 2008). It was also noted that obakunone

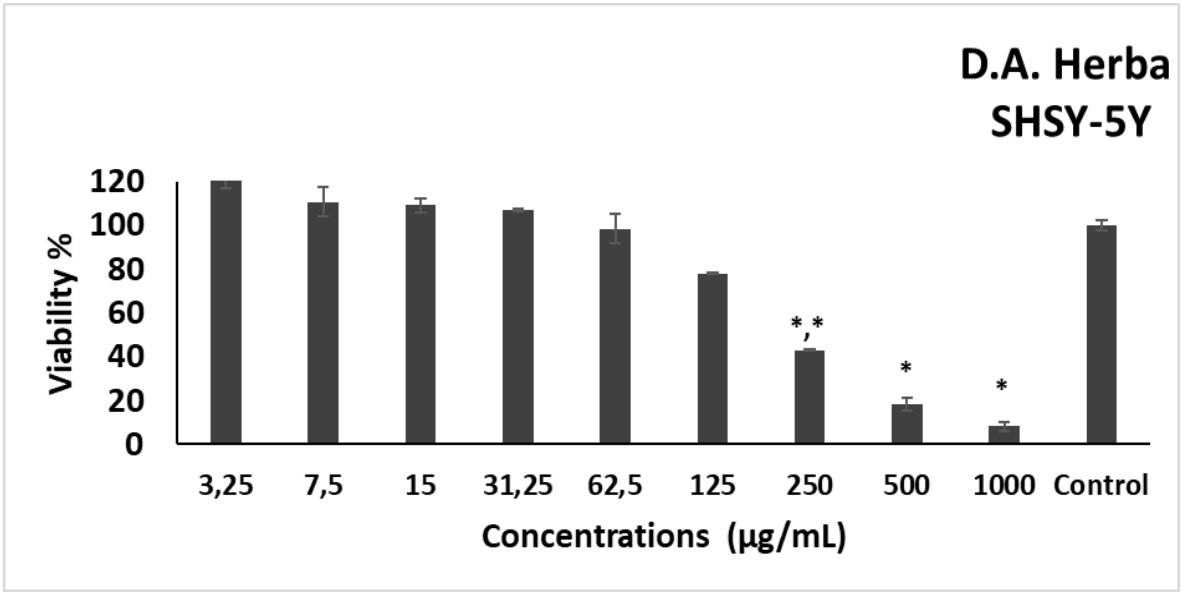

Figure 1. Toxicity profile of D. A herba extract in SHSY-5Y cell line. Values are presented as mean ± standard errors (n = 3), with statistical analysis performed using the Dunnett T3 comparison test.* p<0.001; ** p<0.01.

Şekil 1. *D. A Herba ekstresinin SHSY-5Y hücre hattında toksisite profili. Değerler, Dunnett T3 karşılaştırma testi kullanılarak gerçekleştirilen istatistiksel analiz ile ortalama ± standart hatalar (n = 3) olarak sunulmuştur.* p<0,001; ** p<0.01.*

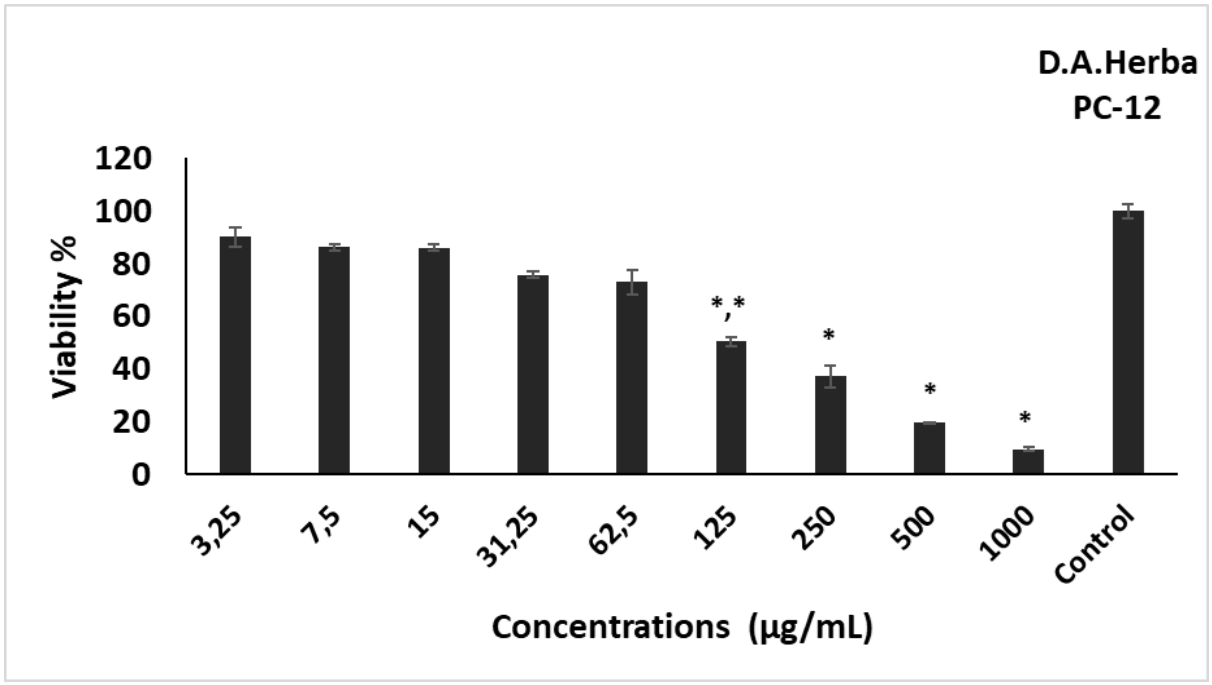

Figure 2. Toxicity profile of D. A Herba extract in a PC-12 cell line. Values are presented as mean ± standard errors (n = 3), with statistical analysis performed using the Dunnett T3 comparison test. * p<0.001; ** p<0.01.

Şekil 2. *D. A Herba ekstresinin PC-12 hücre hattında toksisite profili. Değerler, Dunnett T3 karşılaştırma testi kullanılarak gerçekleştirilen istatistiksel analiz ile ortalama ± standart hatalar (n = 3) olarak sunulmuştur. * p<0,001; ** p<0.01.*

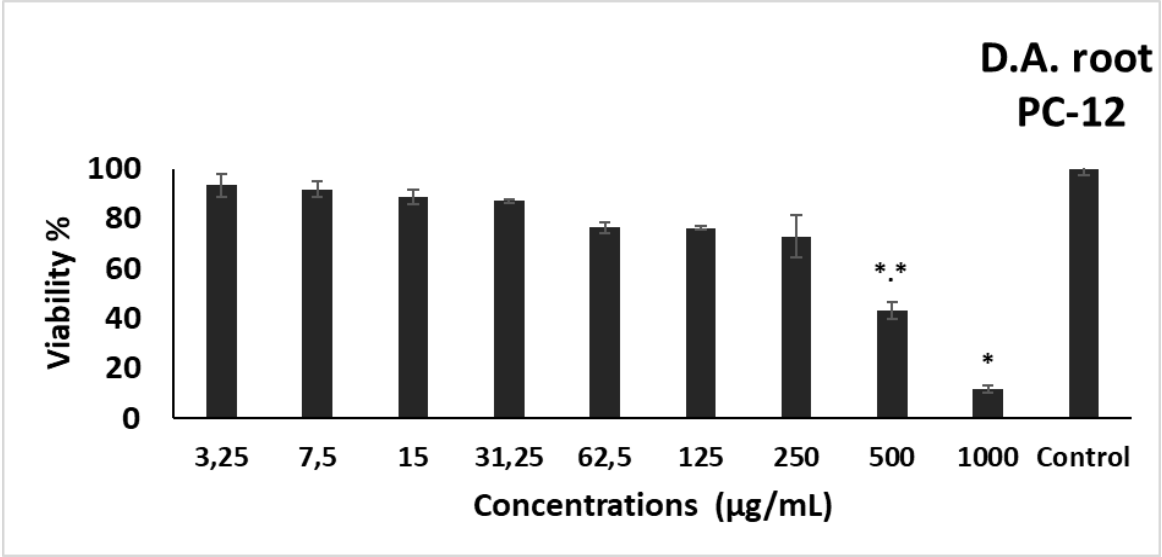

Figure 3. Toxicity profile of D. A Root extract in SHSY-5Y cell line. Values are presented as mean ± standard errors (n = 3), with statistical analysis performed using the Dunnett T3 comparison test. * p<0.001; ** p<0.01; *** p<0.05.

Şekil 3. *D. A Root ekstresinin SHSY-5Y hücre hattında toksisite profili. Değerler, Dunnett T3 karşılaştırma testi kullanılarak gerçekleştirilen istatistiksel analiz ile ortalama ± standart hatalar (n = 3) olarak sunulmuştur. * p<0,001; ** p<0.01.*

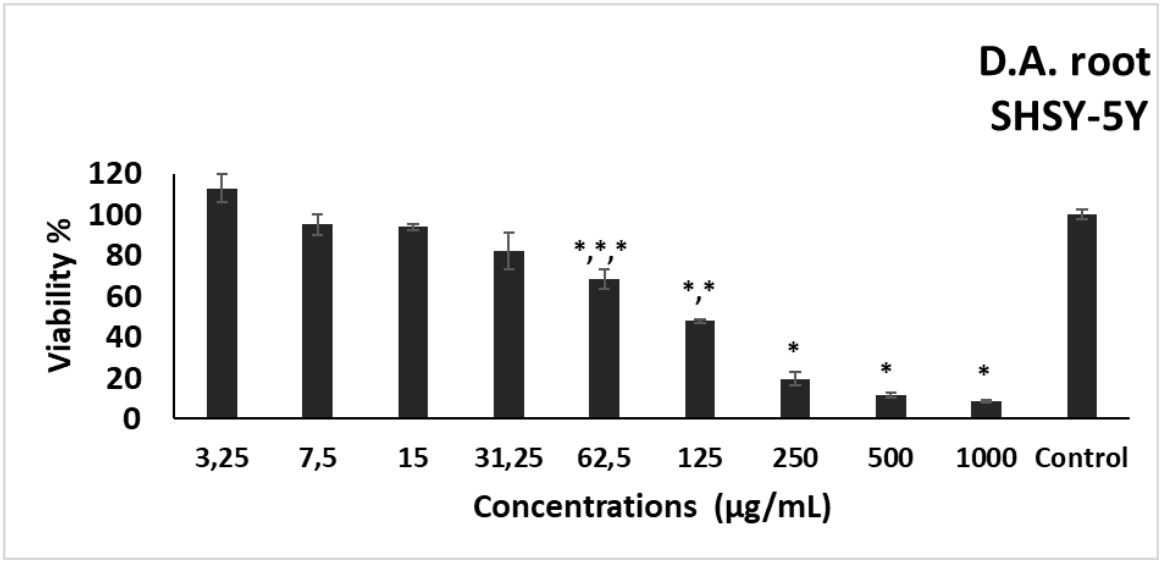

Figure 4. The toxicity profile of D. A Root extract in PC-12 cell line Values is presented as mean ± standard errors (n = 3), with statistical analysis performed using the Dunnett T3 comparison test. * p<0.001; ** p<0.01.

Şekil 4. *D. A Root ekstresinin PC-12 hücre hattında toksisite profili. Değerler, Dunnett T3 karşılaştırma testi kullanılarak gerçekleştirilen istatistiksel analiz ile ortalama ± standart hatalar (n = 3) olarak sunulmuştur. * p<0,001; ** p<0.01.*

Table 5. The IC50 value of D.A. Herba and D.A. Root extracts in SHSY-5Y and PC-12 cell lines were determined by the MTT assay

Çizelge 5. *D.A. Herba ve D.A. Root ekstrelerinin SHSY-5Y and PC-12 hücre hatlarında MTT deneyi ile belirlenen IC50 değerleri*

	IC50 (µg/mL) SHSY-5Y	PC-12
D.A. Herba	171.85±18.22	484.79±5.92
D.A. Root	92.12±2.48	167.26±1.76

isolated from this plant was preventive against glutamate-induced oxidative damage in mouse hippocampal HT22 cells (Jeong et al., 2010).

According to the results, although the extracts did not show a high antioxidant effect compared to the positive control, it was found to be moderately effective depending on the concentration. When the toxicity was evaluated, it was observed that the extracts showed neuroprotective effects on SHSY-5Y and PC-12 cells, especially at decreasing concentrations.

CONCLUSION

This research, which is a preliminary study for *D. albus* growing in Türkiye, is important in terms of determining its neuroprotective and neurotoxic effects as a result of the data obtained. The data will guide the planning of future studies. Thus, *D. albus*, which is a polymorphic species and spread all over the world as a single species, can be compared with taxa grown in other regions. This medicinal plant and its active ingredients can be evaluated in detail.

Acknowledgement

We thank you to Dr. Necmettin Güler for his support in the collection of the plant materials and the diagnosis of the samples.

Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

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