Botanica SERBICA

(2024) 48 (2): 141–149

DOI: https://doi.org/10.2298/BOTSERB2402141S
journal homepage: botanicaserbica.bio.bg.ac.rs

Original Scientific Paper

Determination of the chemical composition, DNA cleavage, binding and antioxidant activities of *Vincetoxicum scandens*

Emine Kılıçkaya Selvi[1*], Seher Güven[2], Nilgün Güler[3] and Kamil Coşkunçelebi[3]

1 Department of Chemistry and Chemical Processing Technologies, Mustafa Çıkrıkçıoğlu Vocational School, Kayseri University, Kayseri, Turkey
2 Department of Biology, Faculty of Sciences and Arts, Recep Tayyip Erdogan University, Rize, Turkey
3 Department of Biology, Science Faculty, Karadeniz Technical University, Trabzon, Turkey
* Correspondence: emineselvi@kayseri.edu.tr

ABSTRACT:
Vincetoxicum members offer important qualities in both conventional and modern medicine. In the present study, *V. scandens* from the northeast of Turkey (Trabzon city) was investigated for its phenolic compounds, antioxidant activities, DNA cleavage and binding effects. Phenolic compounds of the *V. scandens* methanol extract were analysed by high performance liquid chromatography (HPLC-DAD), while their total phenolic and total flavonoid contents were determined spectrophotometrically. The antioxidant potential of the extract was characterised using DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2´-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), and FRAP (ferric reducing antioxidant power assay) tests. In addition, the DNA cleavage and binding features of the *V. scandens* extract were studied using pBR322 DNA and CT-DNA, respectively. The results indicated that the *V. scandens* methanol extract contained a large amount of catechin, as well as a high total phenolic and total flavonoid content. On the other hand, the DPPH and ABTS antioxidant tests revealed that *V. scandens* did not exhibit high antioxidant activity. The methanol extract of the studied plant was also rich in catechin, *p*-coumaric acid and vanillic acid. While the *V. scandens* methanol extract exhibited weak antioxidant and DNA cleavage activity, it showed DNA binding activity at 50 μM, thus demonstrating the potential to be an intercalation agent in this concentration. This study provides the first report on the total phenolic content, total flavonoid content, DNA cleavage and DNA binding activity as well as antioxidant activity of *V. scandens*.

Keywords:
Antioxidant activity, DNA cleavage, HPLC-DAD, *Vincetoxicum scandens*

UDC: 582.923.5:576.3

Received: 20 December 2023
Revision accepted: 17 May 2024

INTRODUCTION

Vincetoxicum Wolf s.l., one of the largest and widespread genera of the subfamily Asclepiadoideae (Apocynaceae), comprises approximately 300 species naturally distributed in a wide region expanding from Europe, Russia and the Caucasus through the Mediterranean, Africa and the Far East via Anatolia (Liede-Schumann & Meve 2018). The genus is also represented in North America with a few introduced species (Sheeley & Raynal 1996). *Vincetoxicum* individuals occupy an important place in both conventional and modern medicine (Zaidi & Crow 2005). Some members of *Vincetoxicum* have been used in the treatment of neurosis, malaria, scrofula, ruptures, injuries, fever, wounds and scabies (Wang *et al.* 2007), while others are reported for their expectorant, diuretic, emetic (Šliumpaite *et al.* 2013), laxative and diaphoretic effects (Ditommaso *et al.* 2005).

Various phytochemical investigations have revealed the presence of phenolics and flavonoids (Šliumpaite *et al*. 2013; Noorian *et al*. 2015, 2016), acetophenones and pregnane glycosides (Lavault *et al*. 1999), alkaloids (Lavault *et al*. 1994), and triterpenoids (Nowak & Kisiel 2000) in *Vincetoxicum* taxa. Moreover, numerous biological activities including anticancer (Lv *et al*. 2012), cytotoxic (Stærk *et al*. 2000, 2002), antibacterial and antifungal (Mogg *et al*. 2008; Gibson *et al*. 2011; Zaidi & Crow 2012), antioxidant (Šliumpaite *et al*. 2013; Noorian *et al*. 2015, 2016), antidiarrheal and antispasmodic (Shah *et al*. 2011), antifeedant (Mogg *et al*. 2008), antileishmanial and antimalarial (Mansoor *et al*. 2011), and anti-inflammatory (Zahra *et al*. 2020) have been substantiated for several *Vincetoxicum* species excluding those of Turkey (e.g. *V. arnottianum* (Wight) Wight, *V. hirundinaria* Medic., *V. lutea* L., *V. nigrum* (L.) Moench, *V. pumilium* Decne., *V. rossicum* (Kleopow) Barbar., *V. stocksii* Ali & Khatoon).

In Turkey, the genus *Vincetoxicum* is represented by 11 taxa, three of which are endemic (Güven *et al*. 2021). From the Turkish *Vincetoxicum*, individuals of three taxa, *V. canescens* (Willd.) Decne., *V. fuscatum* (Hornem.) Rchb. f. and *V. parviflorum* Decne., have recently been studied for their fatty acid, sterol, and tocol compositions, their total phenolic, total flavonoid, amino acid, mineral, glycoside, and sugar contents, and antioxidant, antifeedant (Güzel *et al*. 2017; Güzel 2020), antibacterial, and antifungal activities (Güzel *et al*. 2015, 2018a, b, 2019).

Vincetoxicum scandens Sommier & Levier (Syn. *Antitoxicum scandens* Pobed.), characterised by twining herbaceous stems up to 2 m high and dark blackish-purple coloured flowers with a pilose inner surface, partly fused corona segments, obovate pollinia, and slender ovoid fruits, has a wide distribution in moist forest clearings in northeastern Anatolia (Güven *et al*. 2021). Its native range extends from Eastern Europe (Ukraine and Russia) to Turkey, Caucasia and Northern Iran (Güven *et al*. 2021). There is limited data on the evaluation of the phenolic content and antioxidant activity of *V. scandens*. Only the hydroxycinnamic acid and flavonoid contents of *V. scandens* from the Crimean oblast have previously been studied (Fursa *et al*. 1977).

Since new pharmacologically effective synthetic drugs might cause the emergence of unwanted side effects, phytochemicals are globally considered a safer option. Plants are a major source of antioxidants, which protect the human body against diseases caused by free oxygen derivatives (Chaudhary *et al*. 2023). Herbal medicines have also long been used in the treatment of diseases such as cancer. Recently, the binding and cleavage effects of DNA were determined to be important criteria for the inhibition of cancer (Gurova 2009). Therefore, discovering new plant extracts which include richer phytochemical contents has been of great importance for pharmaceutical raw materials and DNA binding, intercalating agents (Sxena *et al*. 2013; Bačkorová *et al*. 2014; Plsíkova *et al*. 2014; Chavant *et al*. 2015; Koche *et al*. 2016).

Since there is no prior report on the evaluation of the antioxidant activity, DNA cleavage and binding effects of the potential medicinal plant, *V. scandens*, this study was conducted for three purposes: (1) to identify and quantify the phenolic compounds in the methanol extract of *V. scandens*, (2) to evaluate the antioxidant activity of the extract using 2,2-diphenyl-1-picrylhydrazil (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and ferric reducing antioxidant power assay (FRAP) tests, and (3) to determine the DNA cleavage and binding activities of *V. scandens*.

MATERIALS AND METHODS

Chemicals and solvents. Unless otherwise stated, all the analytical grade chemicals and all the phenolic standards as well as CT-DNA (D1501) were purchased from Sigma–Aldrich (St. Louis, MO, USA). The HPLC-grade methanol, acetic acid, Folin-Ciocalteu reagent, sodium carbonate, and sodium hydroxide were supplied by Merck (Darmstadt, Germany). The HPLC syringe filters (RC-membrane, 0.20 µm) were purchased from Sartorius Minisart RC 15, Sartorius (Germany).

Sample preparation. Plant samples of *V. scandens* were collected from Uzungöl, in the forest clearings around the lake, Çaykara-Trabzon in Turkey. The collected plant samples were identified, and deposited at the Herbarium of Recep Tayyip Erdoğan University, Department of Biology with the voucher specimen number S. Güven 169 & S. Makbul. The plant material (roots, stems, leaves, and fruits) used in the experiments was dried at room temperature in the shade, and ground with a blender (Waring Commercial, CT, USA). Five grams of dried samples were added to a flask with 50 mL methanol, and extracted in an ultrasonic bath (Elma Clean Box, Elma) at 40°C for 60 min. The extract was centrifuged for 10 min, at 5000 rpm. 50 mL of the final extract was transferred to a new flask, and evaporated using a rotary evaporator. The dried extract was dissolved in methanol, and stored at -18°C prior to analysis.

Determination of the phenolic compounds by HPLC-DAD. Chromatographic analysis was performed by using an Agilent 1200 series high pressure liquid chromatography (HPLC) system (Agilent Technologies, Mississauga, ON, Canada) equipped with a DAD detector. Chromatographic separation was performed on a C18 column (150 × 4.6 mm i.d., 5 µm particle, 100 A°; Agilent). Gradient elution was used for the HPLC analyses using three mobile phases: A [MeOH:water:acetic acid (10:85:5, v/v/v)], solvent B [MeOH:water:acetic acid (91:8:1, v/v/v)], and solvent C (MeOH). The gradient was

as follows : the composition of solvent B was increased from 12 to 30% over 15 min, then to 42% over 3 min and held for 15 min, and finally to 100% over 5 min, while the composition of solvent C was increased to 12% over 2 min, then to 35% over 11 min and then returned to the initial conditions over 2 min and held for 5 min. The total run time was 55 min. The detection wavelengths were set at 254, 280, 320, and 360 nm. The volume of injection was 20 µL, the flow rate was set at 1 mL min^{-1} and the column temperature was 30°C. Gallic acid, protocatechuic acid, catechin, chlorogenic acid (3-caffeoylquinic acid), 2,5-dihydroxybenzoic acid, caffeic acid, vanillic acid, rutin, p-coumaric acid, ferulic acid, quercetin, kaempferol, and isorhamnetin were used for the phenolic standards. The spectrum was recorded between 200–400 nm.

Determination of the total phenolic content. The total phenolic content of the methanol extract of *V. scandens* was analysed using Folin-Ciocalteu's phenol reagent. The extract was tested at a concentration of 0.5 mg/mL. Briefly, 20 µL plant extract, 680 µL of distilled water, and 400 µL of 0.5 N Folin-Ciocalteu reagents were mixed. After three minutes at room temperature, 400 µL of 10% Na_2CO_3 was added to the mixture. Gallic acid and quercetin were used to generate a standard curve in a range from 0.0019 to 1 mg mL^{-1} ($r^2 = 0.999$) (Singleton *et al.* 1999). All the experiments were performed in triplicate and the absorbance of the mixture was measured at 760 nm with a UV-Vis spectrophotometer (Labomed Inc. Culver City-USA). The concentration of the total phenolic compounds was expressed as mg of gallic acid equivalent (GAE) per g of dry weight (dw), and quercetin equivalent (QE) per g of dw.

Determination of the total flavonoid content. The total flavonoid content was determined by the aluminium complexation method as described by Marcucci *et al.* (1998). In this method, 0.1 mL 10% aluminium nitrate, 0.1 mL 1 M potassium acetate and 4.3 mL 80% ethyl alcohol were mixed with 50 µL of plant extract at a concentration of 1.0 mg/mL. The samples were incubated for 40 min at room temperature, and the absorbance was measured at 415 nm. All the analyses were performed in triplicate using a spectrophotometer (Labomed Inc. Culver City-USA). Quercetin was used as the standard to generate a calibration curve, and the results were expressed as mg quercetin equivalent (QE) per g of dw.

Free radical scavenging activity assay (DPPH). The scavenging activity of the methanol extract against the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined using the spectrophotometric method at 517 nm (Molyneux 2004). The extract was tested at 2.0 mg/mL, and diluted to different concentrations in methanol in the range of 1.00-0.025 mg/mL Briefly, 0.75 mL of plant extract was mixed with 0.75 mL of 0.1 mM DPPH in methanol. The radical scavenging activity was measured using gallic acid and quercetin as the standards. The results were presented as SC_{50} values, indicating the concentration of the sample required to scavenge 50% of DPPH free radicals (SC_{50}; mg sample per mL methanol). All the analyses were performed in triplicate.

ABTS radical scavenging assay. The radical scavenging activity of the extract against ABTS [2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] was spectrophotometrically determined at 734 nm (Benzie & Strain 1999). The ABTS solution was prepared in water at a concentration of 7 mM. The ABTS radical was obtained by reacting the prepared ABTS stock solution with 2.45 mM potassium persulfate and keeping the mixture in the dark at room temperature for 16–18 hours. At 734 nm, the absorbance changed to 0.700 (±0.020). The extract was tested at 2.0 mg/mL, and diluted to different concentrations in methanol in the range of 1.00-0.0125 mg/mL. Afterwards, 20 µL of extract in different concentrations was mixed with 2 mL of the diluted reagent, and after 5 minutes, the absorbance of the mixture was detected at 734 nm. The results were expressed as SC_{50}; (mg sample per mL). All the analyses were performed in triplicate.

Ferric reducing antioxidant power assay (FRAP). The antioxidant capacity of the methanol extract was spectrophotometrically determined using the FRAP assay (Lu *et al.* 2006). The FRAP reagent was prepared by mixing 25 mL of acetate buffer (300 mM, pH 3.6), 2.5 mL of 10 mM 2,4,6-tris(2-pyridyl)-s-triazine solution in 40 mM hydrochloric acid, and 2.5 mL of 20 mM iron(III) chloride hexahydrate solutions. Hence, 3 mL of the FRAP reagent, 100 µL 0.5 mg/mL of the extract and 100 µL of distilled water were added to a flask and vortexed (Heidolph). After an incubation of 4 min at room temperature, the absorbance values were measured at 593 nm. Trolox was used as the standard ($r^2 = 0.999$). The results were given as µmol trolox equivalent per gram of the extract. All the analyses were performed in triplicate.

DNA cleavage experiment. Agarose gel electrophoresis was used to examine the DNA cleavage activity of the *V. scandens* sample. Supercoiled pBR322 plasmid DNA (100 µg) was used without irradiation. Supercoiled pBR322 plasmid DNA was treated with different concentrations of the samples ranging from 50 to 150 µM in 10 % DMSO. All the samples were incubated in PCR tubes at 37°C for 2 hours. Then, the mixtures were loaded on 1% agarose gel with ethidium bromide staining in a TAE buffer (Tris acetic acid-EDTA). Electrophoresis was performed at 75V for 60 minutes. The results were visualised using the BioRad Gel Doc XR system and analysed by the Image Lab Version 4.0.1 Software programme. All the analyses were performed in triplicate.

DNA binding experiment using gel electrophoresis. The interaction of the samples with CT-DNA was studied by using an agarose gel electrophoresis method. Fifty µM solutions of the *V. scandens* sample were prepared in DMSO. Then, a series of samples at a concentration of 50 µM and different concentrations of CT-DNA (0.375–3 mg/mL in ddw) were prepared, and the volume was adjusted to a final volume of 25 µL with a buffer in PCR tubes. The samples were incubated at 37°C for 2 hours. Then, the mixtures were loaded with the buffer on 1% agarose gel with ethidium bromide staining in TAE. The electrophoresis was performed at 75 V for 2 hours. The results were visualised by using the BioRad Gel Doc XR system (Keleş *et al*. 2019). All the analyses were performed in triplicate.

Statistical analysis. The results from the experiments were presented as the mean ± standard deviations of three parallel measurements. The SC_{50} values were calculated from linear regression analysis (Microsoft Excel program for Windows, version 2003). The statistical analysis of the obtained experimental results was carried out in SPSS (Version 16.0, Chicago, IL) using Mann–Whitney U-test and Pearson correlation analyses. Differences of $p < 0.01$ were considered significant.

RESULTS

Identification of the phenolic compounds in the extract. Thirteen phenolic standards were analysed qualitatively and quantitatively in the methanol extract of *V. scandens* using this optimised and validated HPLC-DAD method (Table 1, Fig. 1). The external calibration method using HPLC-DAD was linear for the phenolic standards with coefficients of determination (r^2) of at least 0.999. Rather low detection limits were obtained for all the standards with the highest values for quercetin and kaempferol with LOQ values of 0.216 and 0.236 mg/L, respectively (Table 1). The primary phenolic compounds found in the methanol extract of *V. scandens* were catechin, *p*-coumaric acid, and vanillic acid, followed by caffeic acid, gallic acid, kaempferol, ferulic acid, chlorogenic acid, isorhamnetin, and rutin (Table 2). Protocatechuic acid, 2,5-dihydroxybenzoic acid and quercetin were not detected in the extract.

Total phenolic content (TPC) and total flavonoid content (TFC). The TPC in the methanol extract of *V. scandens* was determined spectrophotometrically. The obtained results for the TPC and TFC in the *V. scandens* methanol extract are presented in Table 3. According to the results, the TPC values obtained in the methanol extract of *V. scandens* were 98.63 mg GAE/g and 58.45 mg QE/g, and TFC was 52.33 mg QE/g.

Antioxidant activity. In order to determine the antioxidant activity, the extract was analysed using radical scavenging (DPPH and ABTS), and reducing power (FRAP) assays, and the results are presented in Table 4. According to the results of the FRAP analysis, the high activity in the methanol extract of *V. scandens* was 202.27 µmol trolox/g ext. When the ABTS and DPPH activities were examined, the *V. scandens* sample showed weak antioxidant activity compared to the positive controls.

CT-DNA binding. The DNA protective effect of the *V. scandens* extract was tested using pBR322 DNA and CT-DNA. When compared to the control group in well 1, although the CT-DNA concentration decreased in wells 2-5, the brightness of the lines did not show a regular decrease. In the fourth well in particular, damping and neutralisation occurred as a result of the binding interaction between DNA and the *V. scandens* sample (Fig. 2). On the other hand, despite the same amount of DNA in the well compared to the control, a brighter line was formed in the 5th well. This indicated that there was binding with electronic interaction between the *V. scandens* sample and the CT-DNA. The high amount of phenolic and flavonoid nucleophilic and electrophilic groups in the chemical structure of the methanol extract of *V. scandens* provided interaction with the negatively charged CT-DNA.

DNA cleavage. The control DNA structure in well 1, whereas the *V. scandens* extract in other wells with different concentrations induced slight cleavage at all concentrations. The *V. scandens* extract caused cleavage from the supercoiled DNA form to the nicked DNA form (from Form I to II). The results are given in Figure 3.

DISCUSSION

In the methanol extract of *V. scandens*, catechin was determined as the primary phenolic compound since it was found in the highest amount. Previous studies on the phytochemical profile of different plant groups have indicated that catechin is an important phenolic component with antioxidant properties eliminating reactive oxygen species (Grzesik *et al*. 2018). Six substances including sinapic acid, ferulic acid, caffeic acid, chlorogenic acid, quercetin, and kaempferol were previously isolated from *V. scandens* (Fursa *et al*. 1977), and these results are similar to ours from the HPLC-DAD analyses (Table 2). In another study, Šliumpaite *et al*. (2013) identified chlorogenic acid, isoquercitrin and apigenin-7-*O*-glucoside in the methanol and acetone extracts of *V. lutea* by using LC-MS methods. Rutin, gallic acid, caffeic acid, apigenin, myricetin and quercetin were detected in the methanol extract of *V. arnottianum*. While rutin was determined as the primary phenolic compound, gallic acid was found in the lowest amounts (Zahra *et al*. 2020).

Table 1. The standard chromatogram values of thirteen individual phenolic compounds

No	Compounds	Mean Retention Time (Minute)	Regression coefficient	Coefficient of variation of Time	Coefficient of variation Area	Limit of detection (ppm)	Limit of quantification (ppm)
1	Gallic Acid	3.97	0.999	0.521	0.176	0.008	0.024
2	Protocatechuic Acid	5.59	0.999	0.685	0.319	0.007	0.021
3	Catechin	6.69	0.999	0.163	0.196	0,005	0.015
4	Chlorogenic Acid	9.01	0.999	0.175	0.167	0.003	0.010
5	2,5-Dihydroxybenzoic Acid	14.02	0.999	0.165	0.096	0.003	0.009
6	Caffeic Acid	14.92	0.999	0.189	0.289	0.008	0.025
7	Vanillic Acid	19.33	0.999	0.589	0.427	0.004	0.012
8	Rutin	20.71	0.999	0.035	0.152	0.005	0.016
9	*p*-coumaric Acid	22.47	0.999	0.129	0.235	0.007	0.020
10	Ferulic Acid	29.81	0.999	0.083	0.635	0.017	0.050
11	Quercetin	36.38	0.999	0.553	0.226	0.072	0.216
12	Kaempferol	36.59	0.999	0.166	0.248	0.079	0.236
13	Isorhamnetin	40.00	0.999	0.159	0.657	0.007	0.020

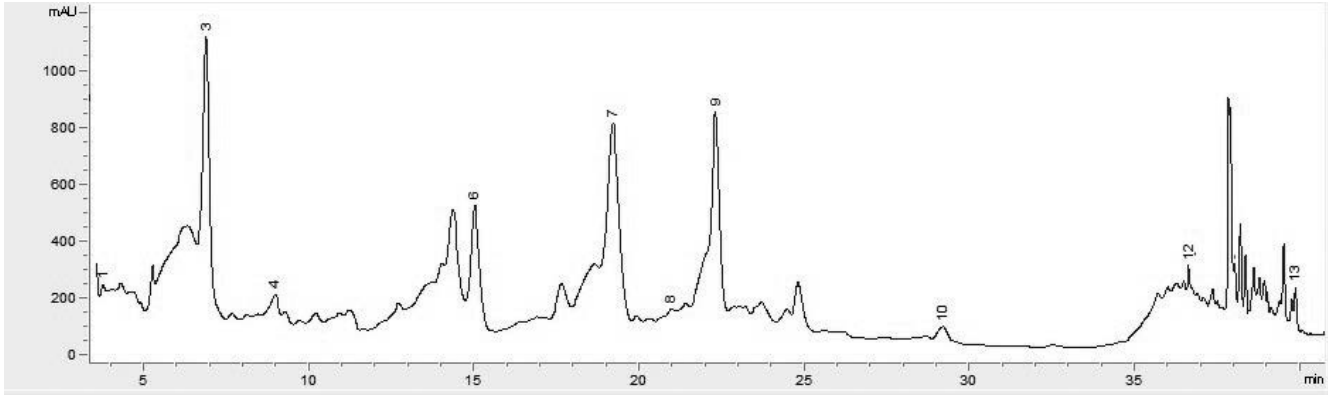

Fig. 1. HPLC–DAD chromatogram of *Vincetoxicum scandens at 280 nm.* 1. Gallic acid, 3. Catechin, 4. Chlorogenic acid, 6. Caffeic acid, 7. Vanillic acid, 8. Rutin, 9. *p*-coumaric acid, 10. Ferulic acid, 12. Kaempferol, 13. Isorhamnetin.

Table 2. The phenolic compounds of the methanol extract of *Vincetoxicum scandens* analysed by HPLC-DAD

Standards	Retention Time Minute	*Vincetoxicum scandens* mg std/g extract
Gallic acid	3.97	1.64
Protocatechuic Acid	-	N.D.
Catechin	6.69	23.76
Chlorogenic acid	9.01	0.42
2.5dihydroxybenzoic acid	-	N.D.
Caffeic acid	14.92	2.08
Vanillic acid	19.33	10.12
Rutin	20.71	0.18
p-coumaric acid	22.47	15.08
Ferulic acid	29.81	0.47
Quercetin	-	N.D.
Kaempferol	36.59	0.85
Isorhamnetin	40.00	0.32

N.D.: Not Detected

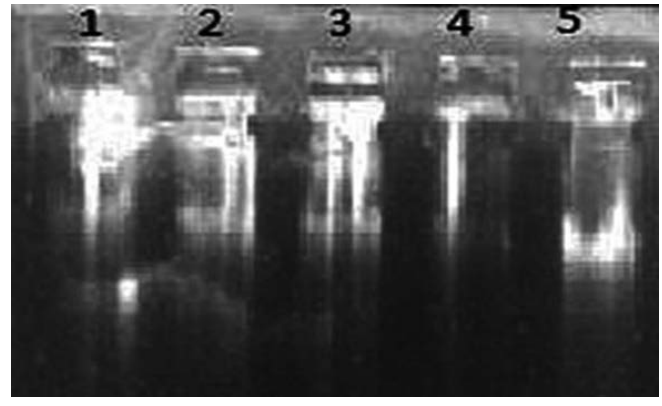

Fig. 2. Electrophoresis gel images of the methanol extract of *Vincetoxicum scandens.* Ingredients of the lanes: Lane 1: CT-DNA (3 mg/mL) in a buffer (Tris HCl, pH=7), Lanes 2-5: CT-DNA (3-0.375 mg/mL) + buffer + *Vincetoxicum scandens* (50 µM).

Table 3. The total phenolic and total flavonoid contents of the extract

	Total phenolic content		Total flavonoid content
Extract	mg GAE/g dw	mg QE/g dw	mg QE/g dw
Vincetoxicum scandens	98.63±0.12	58.45±0.08	52.33±0.09

GAE the equivalent of gallic acid (GA), QE the equivalents of quercetin (Q), dw dried weight

Table 4. The antioxidant activity of the extract

Extract	DPPH, SC_{50} (mg/mL)	ABTS, SC_{50} (mg/mL)	FRAP (μmol trolox/g ext)
Vincetoxicum scandens	0.252±0.031	2.406±0.022	202.27±0.15
Gallic acid	0.002±0.000	0.008±0.000	
Quercetin	0.001±0.000	0.002±0.000	
Trolox	0.002±0.000	0.006±0.000	

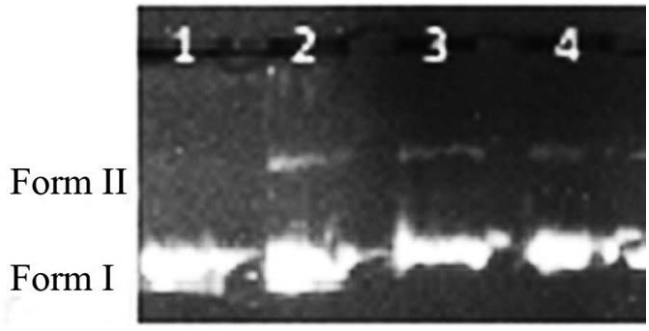

Fig. 3. DNA Cleavage activity gel images of the methanol extract of *Vincetoxicum scandens*. Lane (1) pBR322DNA + 10 μL ddw. Lane (2) pBR 322DNA + 150μg/mL of *Vincetoxicum scandens* + ddw. Lane (3) pBR 322DNA + 100 μg/mL of *Vincetoxicum scandens* + ddw. Lane (4) pBR 322DNA + 50 μg/mL of *Vincetoxicum scandens* + ddw.

This study provides the first report on the TPC and TFC, as well as the antioxidant activity of *V. scandens*, and our data were compared with a limited number of investigations on other *Vincetoxicum* species. Güzel *et al*. (2020) investigated the TPC and TFC of ethanol extracts of *V. canescens* subsp. *canescens* and *V. canescens* subsp. *pedunculata* seeds. The total phenolic and flavonoid contents in their study were 25.62 μg GAE/mg, 16.50 μg GAE/mg and 1.50 μg QE/mg extract, 1.13 μg QE/mg extract, respectively. These findings are not entirely consistent with our result. This difference may be due to the fact that different plant species were used and different solvents were used in the extraction process. Šliumpaite *et al*. (2013) investigated methanol and acetone extracts of *V. lutea* leaves. The TPC in this plant ranged from 86 to 132 mg GAE/dw, and our findings fall within this range. In another study conducted by Slapšytė *et al*. (2019) it was shown that the acetone extract of *V. luteum* had higher TPC levels compared to the acetone and methanol extract of *V. hirundinaria* and also the methanol extract of *V. luteum* (131.8, 127.4, 93.1, and 86.0 mg GAE/g dw, respectively). The TPC results of the methanol extracts of *V. luteum* and *V. hirundinaria* were similar to those obtained in our study. Another study reporting on the TPC and TFC of methanol, dichloromethane and ethyl acetate extracts of *V. nigrum* showed that the methanol extract was richer in TPC and TFC (Nourian *et al*. 2016).

Güzel *et al*. (2020) investigated the antioxidant activity of *V. canescens* subsp. *canescens* and *V. canescens* subsp. *pedunculata* seeds. Butylated hydroxyanisole was used as the standard and the results were calculated using DPPH radical scavenging activity (%). In the case of the DPPH free radical scavenging assay, *V. canescens* subsp. *canescens* showed the highest antioxidant activity of 94.26% at a concentration of 500 μg/mL. Additionally, the antioxidant activity of methanol and acetone extracts of *V. lutea* was confirmed by Šliumpaite *et al*. (2013) using the online HPLC-DPPH method. According to their results, the radical scavenging capacity of the acetone extract was higher than that of the methanol extract with SC_{50} values of 0.13% and 0.21%, respectively. Another study reported the DPPH radical scavenging activity of *V. pumilum* and *V. nigrum* (Nourian *et al*. 2015). According to the results of their study, the methanolic extract of *V. pumilum* exhibited higher antioxidant activity (IC_{50} = 0.21 mg/mL) than *V. nigrum* (IC_{50} = 0.30). These results are compatible with our findings. In addition, Nourian *et al*. (2016) investigated the antioxidant activity of methanol, dichloromethane and ethyl acetate extracts of *V. nigrum* using an online HPLC-DPPH method, with their results indicating that the methanol extract exhibited the highest antioxidant activity (SC_{50} = 1.44 mg/mL). The previous results were lower than the findings obtained in our research.

The most abundant groups of phenolic compounds were phenolic acids (gallic acid, chlorogenic acids, caffeoyl derivative and *p*-coumaroyl derivatives) and flavonols (quercetin and kaempferol and their derivatives).

In our study, when determining the total phenolic content, analyses were carried out on two groups, using gallic acid, which belongs to the phenolic acid group, and quercetin, which is from the flavonol group.

CONCLUSIONS

Plants of the genus *Vincetoxicum* are traditionally used in medicine. In this study, the methanolic extract of *V. scandens* was investigated for the first time in terms of phenolic compounds, antioxidant activities, DNA cleavage and binding effects in order to determine its potential for medicinal usage. As the results of the phytochemical analysis indicate, the methanol extract of *V. scandens* was found to be rich in phenolic compounds and flavonoids. Conversely, *V. scandens* did not exhibit high antioxidant activity in the DPPH and ABTS antioxidant tests. This finding was also supported by the HPLC-DAD analysis. Nevertheless, the results of the HPLC-DAD analysis show that the extract contained a significant amount of catechin with well-reported biological activity potential. Furthermore, the *V. scandens* methanol extract at 50μM demonstrated promising results for evaluation as an intercalating agent due to its strong binding capability with DNA.

REFERENCES

Bačkorová M, Jendželovský R, Kello M, Bačkor M, Mikeš J & Fedoročko P. 2014. Lichen secondary metabolites are responsible for induction of apoptosis in HT-29 and A2780 human cancer cell lines. *Toxicology in Vitro* **26**(3): 462–468.

Benzie I & Strain J. 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology* **299**: 15–27.

Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Abdull Razis AF, Modu B, Calina D & Sharifi-Rad J. 2023. Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. *Frontiers in Chemistry* **11**: 1–24.

Chavant F, Boucher A, Boisselier R, Deheul S & Debruyne D. 2015. New synthetic drugs in addictovigilance. *Thérapie* **70**(2): 179–189.

Ditommaso A, Lawlor FM & Darbyshire SJ. 2005. The Biology of Invasive Alien Plants in Canada. 2. *Cynanchum rossicum* (Kleopow) Borhidi [= *Vincetoxicum rossicum* (Kleopow) Barbar.] and *Cynanchum louiseae* (L.) Kartesz & Gandhi [= *Vincetoxicum nigrum* (L.) Moench]. *Canadian Journal of Plant Science* **85**: 243–263.

Fursa NS, Litvinenko VI & Belyaeva LE. 1977. Hydroxycinnamic acids and flavonoids of *Antitoxicum scandens*. I. *Chemistry of Natural Compounds* **13**: 352.

Gibson DM, Krasnoff SB, Biazzo J & Milbrath L. 2011. Phytotoxicity of antofine from invasive Swallow Worts. *Journal of Chemical Ecology* **37**: 871–879.

Grzesik M, Naparło K, Bartosz G & Bartosz SI. 2018. Antioxidant properties of catechins: Comparison with other antioxidants. *Food Chemistry* **241**: 480–492.

Gurova K. 2009. New hopes from old drugs: revisiting DNA-binding small molecules as anticancer agents. *Future Oncology* **5**(10): 1685–1704.

Güven S, Coşkunçelebi K & Makbul S. 2021. A new *Vincetoxicum* (Apocynaceae) species from Eastern Anatolia an(12): d its relationship based on biosystematic properties. *Nordic Journal of Botany* **39**(12): e03405.

Güzel S. 2020. Fatty acid, sterol, and tocol compositions; amino acid, mineral, total phenolic, and flavonoid contents; and antioxidant activity of seeds of two *Vincetoxicum* taxa. *Chemistry of Natural Compounds* **56**: 202–206.

Güzel S, Pavela R, Ilçim A & Kökdil G. 2017. Phytochemical composition and antifeedant activity of five *Vincetoxicum* taxa against *Spodoptera littoralis* and *Leptinotarsa decemlineata*. *Marmara Pharmaceutical Journal* **21**(4): 872–880.

Güzel S, Pavela R & Kökdil G. 2015. Evaluation of antifungal effect of different polarity extracts from five *Vincetoxicum* taxa against *Aspergillus fumigatus*. *International Anatolian Academic Online Journal/Health Science* **3**(2): 1–9.

Güzel S, Ülger M, Aslan G & Kökdil G. 2018b. Evaluation of antimicrobial activity of five *Vincetoxicum* taxa growing in Turkey. *Marmara Pharmaceutical Journal* **22**(3): 365–373.

Güzel S, Ülger M & Kökdil G. 2018a. Anti(myco)bacterial and antifungal activities of seed pods of four *Vincetoxicum* taxa growing in Turkey. *International Journal of Scientific and Technological Research* **4**(3): 27–38.

Güzel S, Ülger M, Özay Y, Yumrutaş Ö, Bozgeyik İ & Sarikaya Ö. 2019. *Vincetoxicum canescens* subsp. *canescens* ve *Vincetoxicum cancescens* subsp. *pedunculata* Tohumlarının Antimikrobiyal ve Antiproliferatif Aktiviteleri. *Mersin Üniversitesi Tıp Fakültesi Lokman Hekim Tıp Tarihi ve Folklorik Tıp Dergisi* **9**(3): 367–375.

Keleş T, Barut B, Özel A & Biyiklioğlu Z. 2019. Synthesis of water soluble silicon phthacyanine, naphthalocyanine bearing pyridine groups and investigation of their DNA interaction, topoisomerase inhibition, cytotoxic effects and cell cycle arrest properties. *Dyes and Pigments* **164**: 372–383.

Koche D, Shirsat R & Kawale M. 2016. An overerview of major classes of phytochemicals: their types and role in disease prevention. *Hislopia Journal* **9**(1-2): 1-11.

Lavault M, Richomme P & Bruneton J. 1994. New phenantroindolizidine N-oxides alkaloids isolated from *Vincetoxicum hirundinaria* Medic. *Pharmaceutica Acta Helvetiae* **68**(4): 225–227.

Lavault M, Richomme P & Bruneton J. 1999. Acetophenones and new pregnane glycosides from the roots of *Vincetoxicum hirundinaria*. *Fitoterapia* **70**(2): 216–220.

Liede-Schumann S & Meve U. 2018. *Vincetoxicum* (Apocynaceae—Asclepiadoideae) expanded to include *Tylophora* and allies. *Phytotaxa* **369**(3): 129–184.

Lu Z, Nie G, Belton PS, Tang H & Zhao B. 2006. Structure–activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives. *Neurochemistry International* **48**: 263–274.

Lv H, Ren J, Ma S, Xu S, Qu J, Liu Z, Zhou Q, Chen X & Yu S. 2012. Synthesis, biological evaluation and mechanism studies of deoxytylophorinine and its derivatives as potential anticancer agents. *PLoS One* **7**(1): e30342.

Mansoor A, Ibrahim MA, Zaidi MA & Ahmed M. 2011. Antiprotozoal activities of *Vincetoxicum stocksii* and *Carum copticum*. *Bangladesh Journal of Pharmacology* **6**: 51–54.

Marcucci MC, Woisky RG & Salatino A. 1998. Uso de cloreto de alumínio na quantificação de flavonóides em amostras de própolis. *Mensagem Doce* **46**(3): 234–239.

Mogg C, Petit P, Cappuccino N, Durst T, McKague C, Foster M, Yack JE, Arnason JT & Smith ML. 2008. Test of the antibiotic properties of the invasive vine *Vincetoxicum rossicum* against bacteria, fungi and insects. *Biochemical Systematics and Ecology* **36**: 383–391.

Molyneux P. 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant. *Warasan Songkhla Nakharin* **26**: 211–219.

Noorian S, Sani AM, Golmakani E, Feizi P & Roghani K. 2016. Determination of antioxidant activity by high performance liquid chromatography, phenolic and flavonoid contents of *Vincetoxicum nigrum*. *International Journal of PharmTech Research* **9**(3): 150–157.

Noorian S, Sani AM & Yazdani A. 2015. Evaluation of phenolic compounds and DPPH inhibition of two Iran endemic species from *Vincetoxicum* genus. *Asian Journal of Biological and Life Sciences* **4**(1): 35–38.

Nowak R & Kisiel W. 2000. Hancokinol from *Vincetoxicum officinale*. *Fitoterapia* **71**(5): 584–586.

Plsíkova J, Stepankova J, Kasparkova J, Brabec V, Backor M & Kozurkova M. 2014. Lichen secondary metabolites as DNA-interacting agents. *Toxicology in Vitro* **28**(2): 182–186.

Shah AJ, Zaidi MA, Sajjad H & Gilani HAH. 2011. Antidiarrheal and antispasmodic activities of *Vincetoxicum stocksii* are mediated through calcium channel blockade. *Bangladesh Journal of Pharmacology* **6**: 46–50.

Sheeley SE & Raynal DJ. 1996. The distribution and status of species of *Vincetoxicum* in eastern North America. *Bulletin of the Torrey Botanical Club* **123**: 148–156.

Singleton VL, Orthofer R & Lamuela Raventós RM. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, *Methods in Enzymology* **299**: 152–178.

Slapšytė G, Dedonytė V, Adomėnienė A, Lazutka JR, Kazlauskaitė J, Ragažinskienė O & Venskutonis PR. 2019. Genotoxic properties of *Betonica officinalis, Gratiola officinalis, Vincetoxicum luteum* and *Vincetoxicum hirundinaria* extracts. *Food and Chemical Toxicology* **134**: 110815.

Stærk D, Christensen J, Lemmich E, Duus JØ, Olsen CE & Jaroszewski JW. 2000. Cytotoxic activity of some phenanthroindolizidine N-Oxide alkaloids from *Cynanchum vincetoxicum*. *Journal of Natural Products* **63**(11): 1584–1586.

Stærk D, Lykkeberg AK, Christensen J, Budnik BA, Abe F & Jaroszewski JW. 2002. In vitro cytotoxic activity of phenantroindolizidine alkaloids from *Cynanchum vincetoxicum* and *Tylophora tanakae* against drug-sensitive and multidrug-resistant cancer cells. *Journal of Natural Products* **65**(9): 1299–1302.

Sxena M, Jyoti S, Nema R, Dharmendra S & Abhishek G. 2013. Phytochemistry of medicinal plants. *Journal of Pharmacognosy and Phytochemistry* **1**(6): 168–182.

Šliumpaite I, Murkovic M, ZEB A & Venskutonis R. 2013. Antioxidant properties and phenolic composition of swallow-wort (*Vincetoxicum lutea* L.) leaves. *Industrial Crops and Products* **45**: 74–82.

Wang L-Q, Lu Y, Zhao Y-X, Zhou J. & Zheng Q-T. 2007. Three new C21-steroidal glycosides from the roots of *Cynanchum inamoenum*. *Journal of Asian Natural Products Research* **9**(8): 771–779.

Zahra Z, Khan, MR, Shah SA, Maryam S, Majid M, Younis T & Sajid M. 2020. *Vincetoxicum arnottianum* ameliorate inflammation by suppressing oxidative stress and pro-inflammatory mediators in rat. *Journal of Ethnopharmacology* **252**: 112565.

Zaidi MA & Crow Jr SA. 2005. Biologically active traditional medicinal herbs from Balochistan, Pakistan. *Journal of Ethnopharmacology* **96**(1–2): 331–334.

Zaidi MA & Crow Jr SA. 2012. Cytotoxicity of four medicinal plants of Pakistan. *Pakistan Journal of Botany* **44**: 395–397.

REZIME

Botanica SERBICA

Određivanje hemijskog sastava, cepanja DNK, vezivanja i antioksidativnih aktivnosti *Vincetoxicum scandens*

Emine Kılıçkaya Selvi, Seher Güven, Nilgün Güler i Kamil Coşkunçelebi

Predstavnici roda *Vincetoxicum* imaju važnu ulogu kako u tradicionalnoj, tako i u modernoj medicini. U ovoj studiji, *V. scandens* sa severoistoka Turske (grad Trabzon) je istraživan sa aspekta fenolnih jedinjenja, antioksidativnih aktivnosti, cepanja DNK i efekata vezivanja. Fenolna jedinjenja metanolnog ekstrakta *V. scandens* analizirana su tečnom hromatografijom visokih performansi (HPLC-DAD), dok je ukupni sadržaj fenola i ukupnih flavonoida određivan spektrofotometrijski. Antioksidativni potencijal ekstrakta je okarakterisan korišćenjem DPPH (2,2-difenil-1-pikrilhidrazil), ABTS (2,2′-azino-bis (3-etilbenzotiazolin-6-sulfonska kiselina)) i FRAP (test snage fero-redukcionih antioksidansa) testovima. Osim toga, cepanja DNK i karakteristike vezivanja ekstrakta *V. scandens* su proučavani korišćenjem pBR322 DNK i CT-DNK, respektivno. Rezultati su pokazali da metanolni ekstrakt *V. scandens* ima veliku količinu katehina, visok sadržaj ukupnih fenola i ukupnih flavonoida. S druge strane, DPPH, ABTS antioksidativni testovi su otkrili da *V. scandens* nema visoku antioksidativnu aktivnost. Ekstrakt metanola ispitivane biljke imao je velike količine katehina, p-kumarinske kiseline i vanilinske kiseline. Dok je metanolni ekstrakt *V. scandens* imao slabu antioksidativnu i aktivnost cepanja DNK, pokazao je aktivnost vezivanja DNK na 50 μM, odnosno potencijal da bude interkalacioni agens u ovoj koncentraciji. Ova studija daje prvi izveštaj o ukupnom sadržaju fenola, ukupnom sadržaju flavonoida, cepanju DNK i aktivnosti vezivanja DNK, kao i antioksidativnoj aktivnosti *V. scandens*.

Ključne reči: antioksidativna aktivnost, cepanje DNK, HPLC-DAD, *Vincetoxicum scandens*

ORCID:

Emine Kılıçkaya Selvi https://orcid.org/0000-0003-0291-5362
Nilgün Güler https://orcid.org/0000-0003-3022-4876
Seher Güven https://orcid.org/0000-0001-8883-8489
Kamil Coşkunçelebi https://orcid.org/0000-0001-5713-6628