STUDIA UBB CHEMIA, LXIX, 1, 2024 (p. 163-174)
(RECOMMENDED CITATION)
DOI:10.24193/subbchem.2024.1.10

DETERMINATION OF CHEMICAL COMPOSITIONS, ANTIOXIDANT, DNA CLEAVAGE AND BINDING PROPERTIES OF *VINCETOXICUM TMOLEUM* EXTRACT

Emine KILIÇKAYA SELVI[a,*], Nilgün GÜLER[a], Seher GÜVEN[b], Serdar MAKBUL[b]

ABSTRACT. In the present study, phenolic compounds, antioxidant activities, DNA cleavage and binding effects of *Vincetoxicum tmoleum* (Vt) collected from west of Anatolia (Manisa city) were investigated. Antioxidant potentials of the extracts were characterized with their total phenolic and flavonoid contents, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), and FRAP (ferric reducing antioxidant power assay) tests. Besides, the DNA cleavage and binding features of *V. tmoleum* extract were studied using pBR322 DNA and CT-DNA, respectively. Phenolic compounds of the extract were analyzed by high performance liquid chromatography (HPLC-DAD). As a result, *V. tmoleum* methanol extract was found to have the high total phenolic and total flavonoid content and antioxidant effect. Strong positive correlations were also found between DPPH and TFC ($r = 0.995$; $p < 0.01$), and TPC ($r = 0.989$;$p < 0.01$), ABTS ($r = 0.994$;$p < 0.01$) and FRAP($r=0,995$; $p < 0.01$). Methanol extract of *V. tmoleum* had large amounts of *p*-coumaric acid, ferulic acid and protocatechuic acid. While *V. tmoleum* samples showed weak DNA cleavage activity, they showed DNA binding activity at 50 µM concentration, that is, the potential to be an intercalation agent in this concentration.

Keywords: *Vincetoxicum tmoleum, antioxidant activity, DNA cleavage, HPLC-DAD*

[a] Department of Chemistry and Chemical Processing Technologies, Mustafa Çıkrıkcıoglu Vocational School, Kayseri University, Kayseri, Türkiye
[b] Department of Biology, Faculty of Sciences and Arts, Recep Tayyip Erdogan University, Rize, Türkiye
* *Corresponding author e-mail: emineselvi@kayseri.edu.tr*

INTRODUCTION

Vincetoxicum Wolf belonging to the subfamily Asclepiadoideae (Apocynaceae) is represented by approximately 300 species naturally distributed in a wide region covering Far East, Africa, the Mediterranean, Anatolia, Caucasus, Russia and Europe [1], and also a few species introduced in North America [2]. The genus *Vincetoxicum* constitutes an important natural plant source for both traditional folk medicine and modern medicine [3]. Some of these herbs are known to be effective in the treatment of common diseases such as malaria, scrofula, rupture, injuries, fever, wounds and scabies [4], and the others are claimed to exhibit expectorant, diuretic, emetic [5], laxative and diaphoretic effects [6]. The literature on chemical composition of *Vincetoxicum* has verified the presence of phenolics and flavonoids [5, 7, 8], acetophenones and pregnane glycosides [9], alkaloids [10], triterpenoids [11] for the members of the genus. Furthermore, depending on the rich chemical content of the genus, many biological activities such as anticancer [12], cytotoxic [13, 14], antibacterial and antifungal [15, 16, 17], antioxidant [5, 7, 8], antidiarrheal and antispasmodic [18], antifeedant [16], antileishmanial and antimalarial [19], anti-inflammatory [20] have been reported for several *Vincetoxicum* species (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) from different countries, except Türkiye.

Türkiye, with 11 wild *Vincetoxicum* species three of which are endemics, constitutes an important natural distribution area for the genus [21]. According to the literature review, a limited number of *Vincetoxicum* species from Türkiye was investigated from a biochemical perspective. *V. canescens* (Willd.) Decne., *V. fuscatum* (Hornem.) Rchb. f. and *V. parviflorum* Decne., have recently been investigated in phytochemical aspect, and fatty acid, sterol, and tocol compositions, total phenolic, total flavonoid, amino acid, mineral, glycoside and sugar contents [22, 23], and antioxidant [23], antifeedant [22], antibacterial and antifungal activities [24, 25, 26, 27] have been reported for these taxa. Ethnobotanical studies reveal that *V. tmoleum* Boiss. has been used as a folk medicine in the treatment of scabies and fungal infections, especially in the Eastern Anatolia region [28]. This species is characterized by erect stem with crisped pubescent, campanulate, yellowish-green flowers with lanate inner surface, free and triangular corona segments, ovate pollinia, and slender ovoid fruits. The native range of this perennial herb is Türkiye to Northern Iraq and Lebanon [29, 30]. However, the widely distributed *V. tmoleum* which has the potential for medical uses has not been biochemically investigated yet.

Medicinal plants have been widely used in the treatment of diseases since ancient times for their rich secondary metabolite content such as phenolic acids, flavonoids, cinnamic acid derivatives, coumarins, tocopherols and tannins which are accepted as natural sources of antioxidants. It is known that antioxidants protect the human body against diseases caused by free oxygen derivatives [31]. Moreover, binding and cleavage of DNA, the heart of cellular transcription and translation, occurs in the cell throughout the natural process with enzymatic or oxidation processes. In addition, many anticancer drugs aim to trigger cell apoptosis by disrupting the DNA structure [32]. Therefore, revealing the interaction of plant extract with DNA and investigating their antioxidant activity is of great importance for production of new drugs. Hence, the present research aims to determine the phytochemical components and investigate the antioxidant activities, DNA cleavage and binding effects of *Vincetoxicum tmoleum*.

RESULTS AND DISCUSSION

Main compounds in the methanolic extract of *V. tmoleum* consisted of ferulic acid, *p*-coumaric acid, and protocatechuic acid, respectively (Table 1, Figure 1). The amount of remaining ones was determined in the following order, gallic acid, chlorogenic acid, apigenin, kaempferol, quercetin. Nevertheless, caffeine, caffeic acid, vanillic acid, rutin and *o*-coumaric acid were not detected in our analyses (Table 1). Numerous studies have demonstrated that *p*-coumaric acid and ferulic acid are among the most significant phenolic compounds responsible for antioxidant activity by eliminating reactive oxygen [33, 34]. Herein, phenolic compounds of *V. tmoleum* extracts were investigated by HPLC-DAD analyses for the first time, and the obtained results are consistent with the phenolic contents identified for the other taxa of *Vincetoxicum*. In a previous study, the presence of chlorogenic acid, isoquercitrin and apigenin-7-O-glucoside was confirmed in the methanol and acetone extracts of *V. lutea* by using LC-MS methods [5]. Similarly, six substances composed of sinapic acid, ferulic acid, caffeic acid, chlorogenic acid, quercetin and kaempferol were isolated from *V. scandens* [35].

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) in the methanol extracts of *V. tmoleum* were determined spectroscopically. According to the present results, the values of TPC were 58.90±0.50 mg GAE/g ext. and 50.39±0.15 mg QE/g ext., and the amount of TFC was 41.37±0.07 mg QE/g ext (Table 2). This study is the first report on the phenolic compounds and antioxidant activity of *V. tmoleum*. The obtained data were compared with limited investigation including a few *Vincetoxicum*

species. The total phenolic and flavonoid contents of ethanol extracts of *V. canescens* subsp. *canescens* and *V. canescens* subsp. *pedunculata* seeds were recorded by Guzel et al. as 25.62 µg GAE/mg extract, 16.50 µg GAE/mg extract and 1.50 µg QE/mg extract, 1.13 µg QE/mg extract, respectively [23]. In another investigation, Šliumpaite et al. [5] reported the amount of TPC ranging from 86 to 132 mg GAE/dw in methanol and acetone extracts of *V. lutea* leaves. Slapšytė et al. [36] used gallic acid as the standard to measure the amount of TPC in *V. luteum* and *V. hirundinaria* specimens. According to that previous study, the values of TPC determined as 131.8 mg GAE/g dw for *V. luteum* and 127.4 mg GAE/g dw for *V. hirundinaria* in the acetone extracts were higher than the ones measured as 93.1 mg GAE/g dw for *V. hirundinaria* and 86.0 mg GAE/g dw for *V. luteum* in methanol extracts. Another study related to the total phenolic and total flavonoid contents of methanol, dichloromethane and ethyl acetate extracts of *V. nigrum* exposed that the methanol extract was richer in total phenolic and total flavonoids [8].

Antioxidant activity of *V. tmoleum* was determined using radical scavenging (DPPH and ABTS), reducing power (FRAP) assays, and the results of antioxidant assay are given in Table 3. The activities of DPPH, ABTS, and FRAP were 0.489±0.018 (SC_{50} mg/mL), 1.403±0.015 (SC_{50} mg/mL) and 152.23±0.35 µmol trolox/g ext, respectively. Several *Vincetoxicum* taxa have been studied for antioxidant activity. Guzel et al. [21] investigated antioxidant activity of *V. canescens* subsp. *canescens* and *V. canescens* subsp. *pedunculata* seeds. In the case of DPPH free radical scavenging assay, *V. canescens* subsp. *canescens* showed the higher antioxidant activity compared to the other subspecies [23]. In addition, HPLC-DPPH based antioxidant activity of *V. lutea* was confirmed using methanol and acetone extracts by Šliumpaite et al. [5]. The researchers indicated that acetonic extracts (with SC_{50} value of 0.13 ± 0.01 %) of *V. lutea* were stronger antioxidants compared to the methanolic extracts (with SC_{50} value of 0.21 ± 0.01 %). In another study, antioxidant activities of two endemic species from Iran, *V. pumilum* and *V. nigrum*, were evaluated using methanol extracts. According to that study, *V. pumilum* had higher antioxidant activity than *V. nigrum* [7]. Moreover, antioxidant activity of methanol, dichloromethane and ethyl acetate extracts of *V. nigrum* was studied by Nourian et al. [8], using an online HPLC-DPPH method. The highest antioxidant activity (SC_{50}, 1.44 mg/mL) was determined in methanol extract compared to the dichloromethane and ethyl acetate extracts [8].

The DNA protective effects of *Vincetoxicum tmoleum* extracts were tested using pBR322 DNA and CT-DNA. When compared to the control group in well 1, despite the same amount of DNA in the well compared to the control, a brighter line was formed in the second well. This indicates that there is a binding with electronic interaction between the *V. tmoleum* extract

and the CT-DNA (Figure 2). Control DNA structure in well 1, methanol extract of *V. tmoleum* in other wells with different concentrations cleaved at all concentrations, albeit slightly, samples caused cleavage from supercoiled DNA form to nicked DNA form (from Form I to II). The result is given in Figure 3.

In this study, correlation analyses among scavenging activity (DPPH), ABTS, FRAP, TPC, and TFC levels were conducted. The correlation coefficients (r) are shown in Table 4. Significant positive linear correlations (Table 4) were established between TPC and TFC ($r = 0.922$; $p < 0.01$). Strong positive correlations (Table 4) were also found between DPPH and TFC ($r = 0.999$; $p < 0.01$), TPC ($r = 0.989$; $p < 0.01$), FRAP ($r = 0.995$; $p < 0.01$), and ABTS ($r = 0.994$; $p < 0.01$).

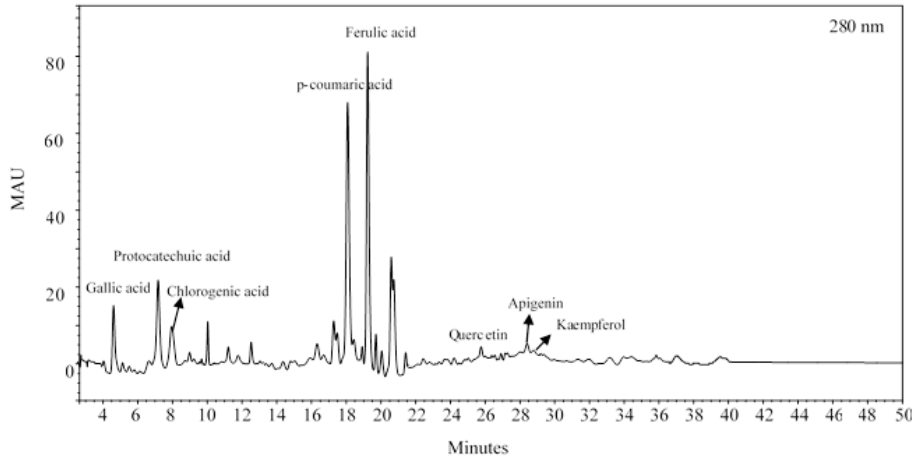

Figure 1. HPLC–DAD chromatogram of methanol extract of *V. tmoleum* at 280 nm.

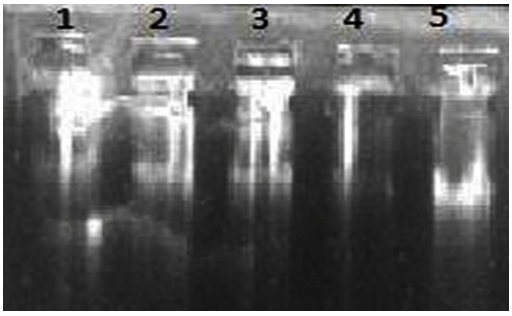

Figure 2. Agarose gel electrophoresis gel images of methanol extract of *V. tmoleum***.** Ingredients of lines: Lane1: CT-DNA (3 mg/mL) in buffer (Tris HCl, pH=7), Lanes 2-5: CT-DNA (3-0.375 mg/mL) + buffer+ *V. tmoleum* (50 µM)

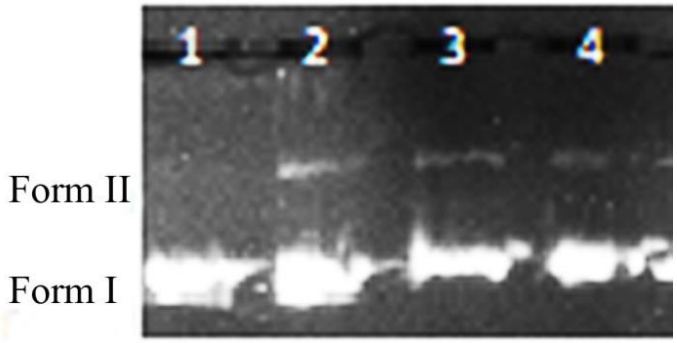

Figure 3. DNA Cleavage activity gel images of methanol extract of *V. tmoleum* Lane (1) pBR322DNA + 10 µL ddw. Lane (2) pBR 322DNA + 150µg/mL of *V. tmoleum* + ddw. Lane (3) pBR 322DNA + 100 µg/mL of *V. tmoleum* + ddw. Lane (4) pBR 322DNA + 50 µg/mL of *V. tmoleum* + ddw.

Table 1. Phenolic compounds of methanol extract of *V. tmoleum* analyzed by HPLC-DAD

Standards	**RT**	***V. tmoleum* mg std/g extract**
Gallic acid	4.56	0.83
Protocatechuic Acid	7.00	1.23
Chlorogenic acid	7.99	0.71
Caffeine	8.80	N.D.
Caffeic acid	10.03	N.D.
Vanillic acid	11.17	N.D
Rutin	12.29	N.D
p-coumaric acid	18.39	3.92
Ferulic acid	19.15	3.94
o-coumaric acid	19.85	N.D.
Quercetin	26.01	0.12
Apigenin	28.47	0.62
Kaempferol	29.00	0.39

N.D.: Not Detected

Table 2. Total phenolic and total flavonoid contents of the extract

	Total phenolic content		**Total flavonoid content**
Extracts	mg GAE/g dw	mg QE/g dw	mg QE/g dw
V. tmoleum	58.90±0.50	50.39±0.15	41.37±0.07

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

Table 3. Antioxidant activity of the methanol extract of *V. tmoleum*

Extract	DPPH, SC_{50} (mg/mL)	ABTS, SC_{50} (mg/mL)	FRAP (µmol trolox/g ext)
V. tmoleum	0.489±0.018	1.403±0.015	152.23±0.35
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	

Table 4. Correlation analysis between TPC, TFC, DPPH, ABTS and FRAP

r	TPC	TFC	DPPH	ABTS
TFC	0.922*			
DPPH	0.989*	0.999*		
ABTS	0.769*	0.957*	0.994*	
FRAP	0.875*	0.994*	0.995*	0.982*

r: Correlation coefficient,
*: Correlation is significant at $p < 0.01$.

CONCLUSIONS

Consequently, in this study, methanolic extract of *V. tmoleum* was investigated for the first time in terms of phenolic compounds, antioxidant activities, DNA cleavage and binding effects in order to determine the probability of medicinal usage. As in the phytochemical analysis results, the methanol extract of *V. tmoleum* was found to be rich in phenolic compounds and flavonoids. This finding was also supported by HPLC-DAD analysis. According to the results of HPLC-DAD analysis, the extract contained significant amount of *p*-coumaric acid and ferulic acid with well-reported biological activity potential.

Significant positive linear correlations were established between TPC and TFC ($r = 0.922$; $p < 0.01$). Strong positive correlations were also found between DPPH and TFC ($r = 0.999$; $p < 0.01$), TPC ($r = 0.989$; $p < 0.01$), FRAP ($r = 0.995$; $p < 0.01$), and ABTS ($r = 0.994$; $p < 0.01$). V*. tmoleum* extract at 50µM concentration was found suitable for evaluation as intercalating agents. The extract performed DNA separation at all concentrations.

EXPERIMENTAL SECTION

Sample Preparation. Plant samples of *V. tmoleum* were collected from Bozdağ, Salihli-Manisa in Türkiye. The collected plant samples were identified and deposited at the Herbarium of Recep Tayyip Erdoğan University, Department of Biology (RUDB) with the voucher specimen number of S. Güven 48 & S. Makbul. All plant samples to be used in the experiments were dried at room temperature in the shade and ground with a blender (Waring Commercial, CT, USA). 5 g of dried samples were added to flask with 50 mL methanol and extracted in the ultrasonic bath (Elma Clean Box, Elma) at 40 ºC for 60 min. Extract was centrifuged for 10 min, at 5000 rpm. The resulting extracts were transferred to a new flask, and the extracts were evaporated using a rotary evaporator. Dried extracts were dissolved in methanol and stored at −18°C until analysis.

Determination of phenolic compounds by HPLC-DAD. Chromatographic analysis was performed by using a Thermo 3600 series high pressure liquid chromatography (HPLC) system equipped with DAD detector. Chromatographic separation was performed on Agilent C18 column (150 × 4.6 mm i.d., 5 µm particle, 100 A°; Agilent). Gradient elution was used for HPLC analyses using two mobile phases as A [Acetic acid:water (2:98 v/v)], solvent B [Acetonitrile:water: (70:30, v/v)], with following gradient: the composition of solvent B was increased from 12 to 25% in 3 min, increased to 45% in 12 min and held for 15 min, and increased to 85% in 10 min, and then returned to the initial conditions in 5 min and held for 10 min. Total run time was 55 min. Detection wavelengths were set at 254, 280, 315, and 360 nm. The volume of injection was 20 µL, the flow rate was set at 0.8 $mL.min^{-1}$ and the column temperature was 30 ℃. Gallic acid, protocatechuic acid, caffeine, vanillic acid, rutin, chlorogenic acid, caffeic acid, *o*-coumaric acid*, p*-coumaric acid, ferulic acid, quercetin, kaempferol, and isorhamnetin were used for phenolic standards.

Methanol, extract of *V. tmoleum* was prepared at a concentration of 2 mg/mL. Stock solutions of the standards were prepared in MeOH at a concentration of 1 mg/mL and then diluted to different concentrations in MeOH in the range of 40–0.5 mg/mL.HPLC analysis of phenolic compounds was detailed by Selvi et al. [43].

Determination of total phenolic content. The total phenolic content of the methanol extract of *V. tmoleum* was analyzed with Folin-Ciocalteu's phenol reagent. Gallic acid and quercetin were used to generate a standard curve in a range from 0.0019 and 1.00 $mg.mL^{-1}$ (r^2 = 0.999) [37]. All experiments were performed in triplicates, and the absorbance of the mixture was measured at 760 nm using UV-Vis spectrophotometer (Labomed Inc.

Culver City-USA). The amount of total phenolic content was given as mg of gallic acid equivalent (GAE) per g of dry weight (dw) and quercetin equivalent (QE) per g dry weight (dw).

Determination of total flavonoid contents. The total flavonoid content was determined by the aluminum complexation method as described by [38]. In this method, 0.1 mL 10% aluminum nitrate, 0.1 mL 1 M potassium acetate, and 4.3 mL 80% ethyl alcohol was mixed with 0.5 mL plant extract. The samples were incubated for 40 min at room temperature, and the absorbance was measured at 415 nm. All analyses were performed in triplicates using 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 dry weight (dw).

Free Radical Scavenging Activity Assay (DPPH). The scavenging activity of methanol extracts against the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined using spectrophometric method at 517 nm [39]. Briefly, 0.75 mL of plant extracts were mixed with 0.75 mL of 0.1 mM DPPH in methanol. Radical scavenging activity was measured using gallic acid and quercetin as standards. Results presented as SC_{50} values indicate the sample of concentration required to scavenge 50% of DPPH free radicals (SC_{50}; mg sample per mL methanol).

ABTS radical scavenging assay. The radical scavenging activity of the extract against ABTS [2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] was spectrophometrically determined at 734 nm [40]. ABTS solution was prepared in water at a concentration of 7 mM. 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. The results were expressed as SC_{50}; (mg sample per mL).

Ferric Reducing Antioxidant Power Assay (FRAP). The antioxidant capacity of the methanol extracts was spectrophometrically determined using FRAP assay [41]. The FRAP reagent was prepared by mixing 25 mL of acetate buffer (300 mM, pH 3.6), 2.5 mL of 10 mM TPTZ solution in 40 mM HCl, and 2.5 mL of 20 mM $FeCl_3{,}6H_2O$ solutions. Trolox was used as a standard ($r^2 = 0.999$). Results were given as µmol trolox equivalent per gram of the extract.

DNA Cleavage experiments. An agarose gel electrophoresis was applied to examine the DNA cleavage activity of Vt samples. Supercoiled pBR322 plasmid DNA (100µg) was used without irradiation. Supercoiled pBR322 plasmid DNA was treated with different concentrations of samples ranging from 50 to 150µM in 10 % DMSO. All 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 TAE buffer (Tris acetic acid-

EDTA). Electrophoresis was performed at 75V for 60 minutes. The results were visualized using the BioRad Gel Doc XR system and analyzed by applying the Image Lab Version 4.0.1 Software program.

DNA Binding experiments using gel electrophoresis. The interaction of the samples with CT-DNA was studied by performing an agarose gel electrophoresis method. 50µM solutions of samples (Vt) were prepared in DMSO. Then, a series of samples containing 50µM and different concentrations of CT-DNA (0.375-3 mg/ml in ddw) were prepared, and the total volume was adjusted to a final volume of 25µl with 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 visualized by applying the BioRad Gel Doc XR system [42].

Statistical Analysis. Results from experiments are 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). Statistical analysis of the experimental results obtained 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.

REFERENCES

1. S. Liede-Schumann; U. Meve; *Phytotaxa*, **2018**, *369*, 129–184.
2. S. E. Sheeley; D. J. Raynal; *Bull. Torrey Bot. Club*, **1996**, *123*, 148–156.
3. M. A. Zaidi; Jr. S. A. Crow; *J. Ethnopharmacol*., **2005**, *96*, 331–334.
4. L. Q. Wang; J. H. Wang; Y. M. Shen; J. Zhou; *Chin. Chem. Lett.,* **2007**, *18*, 1235–238.
5. I. Šliumpaitė; M. Murkovic; A. Zeb; P. R. Venskutonis; *Ind. Crops Prod.,* **2013**, *45*, 74–82.
6. A. DiTommaso; F. M. Lawlor; S. J. Darbyshire; *Can. J. Plant Sci.*, **2005,** *85*, 243–263.
7. S. Noorian; A. M. Sani; A. Yazdani; *Asian J. Biol. Life Sci.*, **2015**, *4(1)*, 35–38.
8. S. Nourian; A. M. Sani; E. Golmakani; P. Feizi; K. Roghani; *Int. J. PharmTech Res.*, **2016**, *9(3)*, 150–157.
9. M. Lavault; P. Richomme; J. Bruneton; *Fitoterapia*, **1999**, *70*, 216–220.
10. M. Lavault; P. Richomme; J. Bruneton; *Pharm. Acta Helv.*, **1994**, *68*, 225–227.
11. R. Nowak; W. Kisiel; *Fitoterapia*, **2000**, *71*, 584–586.
12. U. Tanner; W. Wiegrebe; *Arch. Pharm. (Weinheim)*, **1993**, *326*, 67–72.

13. D. Staerk; J. Christensen; E. Lemmich; J. Duus; C. E. Olsen; J. W. Jaroszewski; *J. Nat. Prod.*, **2000**, *63*, 1584–1586.
14. D. Staerk; A. L. Lykkeberg; J. Christensen; B. A. Budnik; F. Abe; J. W. Jaroszewski; *J. Nat. Prod.*, **2002**, *65*, 1299–1302.
15. M. A. Zaidi; S. A. Crow; *Pak. J. Bot*., **2012**, *44(SI)*, 395–397.
16. C. Mogg; P. Petit; N. Cappuccino; T. Durst; C. McKague; M. Foster; J. E. Yack; J. T. Arnason; M. L. Smith; *Biochem. Syst. Ecol.*, **2008**, *36*, 383–391.
17. D. M. Gibson; S. B. Krasnoff; J. Biazzo; L. Milbrath; *J. Chem. Ecol.*, **2011**, *37*, 871– 879.
18. A. J. Shah; M. A. Zaidi; H. Sajjad; H. A. H. Gilani; *Bangladesh J. Pharmacol.*, **2011**, *6*, 46–50.
19. A. Mansoor; M. A. Ibrahim; M. A. Zaidi; M. Ahmed; *Bangladesh J. Pharmacol.*, **2011**, *6*, 51–54.
20. Z. Zahra; M. R. Khan; S. A. Shah; S. Maryam; M. Majid; T. Younis; M. Sajid; *J. Ethnopharmacol.*, **2020**, *252*, 112565.
21. S. Güven; K. Coşkunçelebi; S. Makbul; *Nord. J. Bot.*, **2021**, *39(12)*, e03405.
22. S. Güzel; R. Pavela; A. İlçim; G. Kökdil; *Marmara Pharm. J.*, **2017**, *21(4)*, 872–880.
23. S. Guzel; *Chem. Nat. Compd.*, **2020**, *56(2)*, 202–206.
24. S. Güzel; R. Pavela; G. Kökdil; *International Anatolian Academic Online Journal/Health Science*, **2015**, *3(2)*, 1–9.
25. S. Guzel; M. Ulger; G. Kokdil; *International Journal of Scientific and Technological Research*, **2018**, *4(3)*, 27–38.
26. S. Güzel; M. Ülger; G. Aslan; G. Kökdil; *Marmara Pharm. J*., **2018**, *22(3)*, 365–373.
27. S. Güzel; M. Ülger; Y. Özay; Ö. Yumrutaş; İ. Bozgeyik; Ö. Sarıkaya; *Mersin Üniversitesi Tıp Fakültesi Lokman Hekim Tıp Tarihi ve Folklorik Tıp Dergisi*, **2019**, *9(3)*, 367–375.
28. E. Altundağ; M. Öztürk; *Procedia Soc. Behav. Sci.*, **2011**, *19*, 756–777.
29. K. Browicz; Asclepiadaceae, in Flora of Turkey and the East Aegean Islands, Vol. 6, P.H. Davis, Ed.; Edinburgh University Press, Edinburgh, UK, **1978**, pp 163-174.
30. S. Güven; S. Makbul; K. Coşkunçelebi; *Recep Tayyip Erdoğan Üniversitesi Fen ve Mühendislik Bilimleri Dergisi*, **2021**, *2 (2)*, 1–12.
31. A. Rice-Evans; N. Miller; G. Paganga; *Trends Plant Sci.*, **1997**, *2(4)*, 152–159.
32. S. Kawanishi; Y. Hiraku; *Curr. Med. Chem. Anticancer Agents*, **2004**, *4(5)*, 415–419.
33. Y. Shen; X. Song; L. Li; J. Sun; Y. Jaiswal; J. Huang; C. Liu; W. Yang; L. Williams; H. Zhang; Y. Guan; *Biomed Pharmacother,* **2019,** 111, 579-587.
34. M. Srinivasan; A. R. Sudheer; M. P. Menon; *J. Clin. Biochem. Nutr.*, **2007**, *40*, 92-100.
35. N. S. Fursa; V. I. Litvinenko; L. E. Belyaeva; *Khlmiya Prirodnykh Soedinenii,* **1977**, *3*, 416.
36. G. Slapšytė; V. Dedonytė; A. Adomėnienė; J. R Lazutka; J. Kazlauskaitė; O. Ragažinskienė; P. R. Venskutonis; *Food Chem. Toxicol.*, **2019**, *134*, 110815.

37. V. L. Singleton; R. Orthofer; R. M. Lamuela Raventós; *Methods Enzymol.,* **1999**, *299,* 152-178.
38. M. Marcucci; R. Woisky; A. Salatino; *Mensagem Doce*, **1998**, *46*, 3-9.
39. P. Molyneux; *Warasan Songkhla Nakharin,* **2004**, *26,* 211–219.
40. I. F. F. Benzie; J. J. Strain; *Methods Enzymol*., **1999**, *299*, 15–27.
41. Z. Lu; G. Nie; P. S. Belton; H. Tang; B. Zhao; *Neurochem. Int.*, **2006**, *48*, 263–274.
42. T. Keleş; B. Barut; A. Özel; Z. Biyiklioglu; *Dyes Pigments*, **2019**, *164*, 372.
43. E. K. Selvi; H. Turumtay; A. Demir; E. A. Turumtay; *Anal. Lett.*, **2018**, *51*, 1464-1478.