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Phenolic profile, antioxidant, antidiabetic, DNA protection, and cytotoxic activities of *Allium kurtzianum*

[1]*Yilmaz-Ozden, T., [1]Hasbal-Celikok, G., [1]Aksoy-Sagirli, P., [1]Altiparmak-Ulbegi, G., [2]Kocyigit, M.,[1]Can, A. and [1]Akev, N.

[1]*Department of Biochemistry, Faculty of Pharmacy, Istanbul University, 34116 Beyazit, Turkey*
[2]*Department of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University, 34116 Beyazit, Turkey*

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Abstract

Allium kurtzianum, known as wild garlic in Turkey, is an endemic and bulbous herb of the Amaryllidaceae family. The antioxidant activities of the aerial parts and bulbs of *A. kurtzianum* were determined using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) assays, along with the determination of total phenolic and flavonoid compounds in the extracts. Also, phytochemical screening of the extracts was performed by using the LC–MS/MS method. DNA damage protection capacity of the extracts was determined using DNA nicking assay. The cytotoxic activity of the extracts was identified using different cancer cell lines. The antidiabetic activity of the extracts was analysed by evaluating inhibitory capacities on α-glucosidase and α-amylase. The results showed that total phenolic and flavonoid compounds of the aerial parts were higher than that of the bulbs. Of the 20 compounds quantified, rutin and fumaric acid were the most abundant phenolic compounds in aerial parts and bulbs extracts, respectively. The antioxidant activity of the aerial parts was greater than that of the bulbs. The extracts also dose-dependently protected pBR322 DNA against the harmful effects of hydroxyl radicals. The aerial parts of *A. kurtzianum* exhibited a cytotoxic effect against prostate, lung, and endometrial cancer cell lines, whereas bulbs of *A. kurtzianum* did not show cytotoxic effect. However, no valuable inhibitory effect on α-glucosidase and α-amylase was observed with either extract. In summary, a methanolic extract from *A. kurtzianum* showed potent antioxidant and DNA protecting effects, as well as anticancer activity against some cancer cells *in vitro*. The phenolic compounds might be responsible for these activities of the plant.

Introduction

Oxidative stress is a condition resulting from an imbalance between oxidant and antioxidant systems, which causes free radical damage (Sies, 2018). Fruits and vegetables are important sources of antioxidants, and they contain potential protective agents against diseases. It is widely believed that diets rich in fruits and vegetables play a crucial role in the prevention of oxidative stress-related human diseases such as cancers (Halliwell and Gutteridge, 2015). When oxidative stress occurs, numerous intracellular molecules are damaged including DNA, RNA, lipids, and proteins. DNA oxidation via reactive species generates a product, 8-hydroxy-2′-deoxyguanosine, that can cause DNA mutations (Sosa *et al.,* 2013). Also, a diet rich in fruits and vegetables is associated with a decreased incidence of type 2 diabetes. In particular, it has been demonstrated that polyphenols in plants may affect blood glucose control through the inhibition of digestive enzymes involved in starch degradation (Boath *et al.,* 2012). The finding of new natural and biologically active compounds from plants that can be used to treat diseases is still an interesting research area.

The genus *Allium* (Amaryllidaceae) is the largest genus in Turkey (Koçyiğıt *et al.,* 2016). *Allium* species have been consumed as food sources, and used as traditional medicines for the treatment of haemorrhoids, earache, diabetes, poisoning, wounds and boils, hypertension, and liver disorders (Kültür, 2007; Asadi-Samani *et al.,* 2015). Previous studies revealed that many *Allium* species possess insecticidal, antimicrobial, antioxidant, antidiabetic, anti-inflammatory, antihypertensive, antimicrobial, anti-atherosclerotic, hepatoprotective, and anticancer properties (Meriga *et al.,* 2012; Asadi-Samani *et al.,* 2015; Hussein *et al.,* 2017).

Allium kurtzianum Asch. and Sint. Ex Kollmann is an endemic plant found on the slopes of

*Corresponding author.
Email: tugbay@istanbul.edu.tr

Kazdagi (Mount Ida) in Turkey (Ozturk *et al.,* 2011). The aim of the present work was to investigate the antioxidant, DNA protection, anticancer, and antidiabetic activities of the aerial parts and bulbs of *A. kurtzianum*. In addition, the phenolic profile of *A. kurtzianum* was also identified by LC-MS/MS method. To the best of the authors' knowledge, the present work is the first study on the phenolic content and biological activities of *A. kurtzianum*.

Materials and methods

Chemicals

α-amylase, α-glucosidase, α-tocopherol, 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 3,5-dinitrosalicylic acid (DNS), acarbose, Folin-Ciocalteu reagent, phenazine methosulfate (PMS), gallic acid, *p*-nitrophenyl α-D-glucopyranoside (*p*NPG), and starch were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Catechin and quercetin were purchased from Fluka Chemical Co. (Buchs, Switzerland). 2,4,6-tripyridyl-s-triazine (TPTZ) was purchased from Merck Chemical Co. (Darmstadt, Germany). pBR322 plasmid was purchased from Thermo Fisher Scientific (Waltham, Massachusetts, USA). 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) was purchased from Promega (WI, Madison, USA). All other chemicals were of analytical grade, unless otherwise stated.

Plant material

The plant material was collected from Kazdagi, Turkey, in August 2014, and was botanically identified by Assoc. Prof. Mine Kocyigit. The voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy, Istanbul University (ISTE-86115).

Preparation of extracts

The aerial parts and bulbs of *A. kurtzianum* were dried in the shade, then ground into powder using a grinder (IKA, Staufen, Germany). The extracts of aerial part and bulb were prepared from 6.76 and 5.48 g of dried plant material, respectively. The ground plant parts were extracted in a Soxhlet apparatus using methanol until the extractive became colourless. The solvents were evaporated (Buchi, Switzerland) until dry under reduced pressure. The methanol extracts were obtained with yields of 15.19% for aerial parts and 6.22% for bulbs.

Determination of total phenolic and flavonoid compounds

The total phenolic and flavonoid contents of the extracts were calculated using Folin-Ciocalteu and aluminium chloride methods, respectively, as described previously (Hasbal *et al.,* 2015). Total phenolic contents of the extracts were expressed in terms of mg gallic acid equivalents (GAE)/g extract, using the equation of a regression curve ($y = 0.4446x + 0.0209$) obtained from standard gallic acid solutions (range of 0.015 - 1 mg/mL). Total flavonoid contents of the extracts were expressed as mg catechin equivalents (CE)/g extract using a regression curve ($y = 1.4787x + 0.0019$) obtained from standard catechin solutions (range of 0.016 - 0.25 mg/mL).

Determination of phenolic profile by LC-MS/MS analysis

The phenolic compounds of the extract from *A. kurtzianum* were determined with an ultra-high performance liquid chromatography (UHPLC) system (UltiMate 3000, Thermo), coupled to a Thermo ORBITRAP Q-EXACTIVE mass spectrometer (MS) equipped with an electrospray ionisation (ESI) source. The extracts were dissolved in 1.5 mL of methanol, and the mixture was centrifuged for 5 min, and the supernatant was transferred into a tube. Then, the internal standard solution (100 mg/L) was added so that the final concentration of the solution was 3 ppm. The final solution was filtered (pore size, 0.45 µm) and injected to the device (Gülçin *et al.,* 2010). Chromatographic separations were performed using a Fortis C_{18} column (150 × 3.0 mm, 3 µm) (Fortis Technologies Ltd., UK) at 25°C. The injection volume was 1 µL. The analytes were eluted employing a gradient program with 1% formic acid in water (A), and 1% formic acid in methanol (B), as the mobile phase at a flow rate of 0.35 mL/min. The gradient program began at 50% of eluent B flow from 0 to 1 min. A linear gradient was applied from 1 to 3 min, from 50 to 100% of eluent B flow, which was maintained for 3 min. Then the eluent B flow was set to its original percent in 1 min, and the system was maintained for 3 min for stabilisation before the next injection. Full scan MS using (+) and (-) ESI modes were acquired in the mass range of 85 - 1500 m/z. The optimised conditions were as follows: sheath gas flow rate, 45 mL/min; auxiliary gas flow rate, 10 mL/min; spray voltage, 3.80 kV; capillary temperature, 320°C; auxiliary gas heater temperature, 320°C; and S-lens RF level, 50.0. The quantification was performed by the external standard method. Calibration curves were plotted from repeating analyses using the linear regression model of least squares. The analytical performance of the

LC-MS/MS method is given in Table 1.

Determination of antioxidant activity

The antioxidant activity of the extracts was measured using DPPH free radical and ferric reducing antioxidant power (FRAP) methods (Hasbal *et al.,* 2015). Quercetin and α-tocopherol were used as standard antioxidants, and a mixture without inhibitor was used as a control. The decrease in the absorbance was measured at 517 nm against a methanol blank. DPPH scavenging activity (%) of the extracts was calculated using Eq. 1. The FRAP values were determined using the equation of the standard regression curve (y = 0.5816x + 0.0197) obtained by $FeSO_4.7H_2O$ (0.2 - 1.5 mM).

$$\text{DPPH scavenging activity, \%} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \quad \text{(Eq. 1)}$$

Determination of antidiabetic activity

The measurement of the antidiabetic activity of the extracts was carried out using α-amylase and α-glucosidase inhibition assays (Hasbal *et al.,* 2017). Acarbose was used as a standard antidiabetic drug, and a mixture without inhibitor was used as a control. The α-amylase inhibitory activity was determined by measuring the absorbance at 540 nm. To determine the α-glucosidase inhibitory activity, the absorbance change at 405 nm was recorded for 10 min at 37°C. The percentage of inhibitory activities of the extracts against α-amylase and α-glucosidase were determined using Eq. 2.

$$\text{Inhibition level, \%} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \quad \text{(Eq. 2)}$$

Determination of DNA protection activity (DNA nicking assay)

The protection activity of the extracts against Fenton's reaction-induced oxidative DNA damage was performed using supercoiled pBR322 DNA following the method of Lee *et al.* (2002). The forms of pBR322 DNA were visualised using Fusion FX (Vilber Lourmat, France), and DNA breakages as percentages were quantified using the Bio1D software

Table 1. Quantitative determination (mg/g extract) of the phytochemicals present in the methanolic extracts of *A. kurtzianum*.

Compound	Amount (mg/g extract)		RT	Linear regression equation	m/z	R^2	Linear range (ppm)	LOD LOQ	Ion mode	U%*
	Aerial part	Bulb								
(+)-Catechin	0.03	0.01	1.79	y = 1.291e-2x + 2.07e-3	289.0718	0.992	0.1 - 7	0.22/0.73	Neg	1.84
(-)-Epicatechin	< LOD	< LOD	2.30	y = 4.593e-3x + 3.546e-4	291.0863	0.990	0.1 - 7	0.23/0.76	Neg	3.61
(-)-Epigallocatechin	0.13	0.19	1.90	y = 8.763e-3x + 8.107e-4	305.0666	0.999	0.1 - 7	0.12/0.40	Neg	3.11
(-)-Epigallocatechin gallate	< LOD	0.01	2.25	y = 3.389e-3x + 2.313e-4	457.0776	0.997	0.1 - 7	0.18/0.59	Neg	2.73
Acacetin	0.07	< LOD	7.07	y = 1.867e-2x - 1.874e-3	283.0612	0.998	0.1 - 7	0.13/0.42	Neg	1.50
Apigenin 7-glucoside	0.04	< LOD	4.07	y = 2.935e-3X + 2.157e-4	433.1129	0.996	0.1 - 7	0.18/0.60	Pos	3.13
Caffeic acid	0.24	0.34	3.30	y = 1.68e-2x + 5.922e-3	179.0350	0.999	0.1 - 7	0.19/0.62	Neg	2.41
Chrysin	< LOD	< LOD	6.40	y = 2.735e-2x - 1.414e-3	253.0506	0.996	0.1 - 7	0.21/0.69	Neg	1.19
Fumaric acid	4.69	5.92	2.36	y = 1.855e-3x + 5.312e-4	115.0037	0.997	0.1 - 7	0.26/0.88	Neg	3.15
Herniarin	0.04	< LOD	4.89	y = 2.041e-1x + 5.651e-2	177.0546	0.998	0.1 - 7	0.17/0.58	Pos	0.94
Hispidulin	0.01	< LOD	5.89	y = 9.085e-3x + 1.581e-3	299.0561	0.997	0.1 - 7	0.14/0.46	Neg	1.73
Hyperoside	13.33	0.07	4.60	y = 2.326e-3x - 2.487e-4	463.0882	0.989	0.1 - 7	0.33/1.09	Neg	3.01
Luteolin-7-rutinoside	1.58	< LOD	4.12	y = 5.179e-3x + 8.77e-4	593.1512	0.997	0.1 - 7	0.22/0.73	Neg	1.43
Naringenin	0.01	< LOD	6.59	y = 1.08e-2x + 1.351e-3	271.0612	0.997	0.1 - 7	0.20/0.67	Neg	4.15
Nepetin	0.21	0.04	6.01	y = 5.633e-2x + 8.265e-3	315.0510	0.997	0.1 - 7	0.12/0.40	Neg	3.21
Nepetin-7-glucoside	2.87	< LOD	4.95	y = 7.781e-3x + 2.388e-3	477.1038	0.996	0.1 - 7	0.22/0.74	Neg	4.39
Quercetin	0.31	0.02	5.42	y = 3.326e-2x + 5.001e-3	301.0354	0.998	0.1 - 7	0.16/0.54	Neg	1.30
Quercitrin	0.86	< LOD	4.95	y = 1.299e-2x + 3.582e-3	447.0932	0.998	0.1 - 7	0.13/0.44	Neg	4.78
Rhamnocitrin	0.03	< LOD	6.21	y = 4.362-3x + 6.084-6	299.0561	0.998	0.1 - 7	0.16/0.53	Neg	2.76
Rutin	32.46	0.36	4.48	y = 2.365e-3x + 7.711e-4	609.1461	0.993	0.1 - 7	0.25/0.85	Neg	4.47

LOD: limit of detection; LOQ: limit of quantification; RT: retention time; Neg: negative; Pos: positive; *Uncertainty (at 95% confidence level, k = 2).

program.

Determination of cytotoxic activity

Human prostate (ATCC CRL-1435, PC-3), human lung (ATCC CCL-185, A549), and human endometrial (ATCC CRL-2923, ECC-1) cancer cell lines were used to evaluate the cytotoxic activity of the extracts. PC-3 cells were grown in DMEM-F12 medium, supplemented with 10% heat-inactivated foetal bovine serum (FBS), 100 units/mL of penicillin, and 100 µg/mL streptomycin. A549 cells were grown in DMEM medium, supplemented with 10% FBS, 100 units/mL of penicillin, and 100 µg/mL streptomycin. ECC-1 cells were grown in RPMI 1640 medium, supplemented with 10% FBS, 100 units/mL of penicillin, 100 µg/mL streptomycin, and 2 mM L-glutamine. All cultures were maintained in an incubator (N-BIOTEK, Korea) at 37°C in a humidified 5% CO_2 atmosphere.

The cytotoxic activity of the extracts was assayed by the "Viability Test" using MTS, according to the manufacturer's instructions (Promega, Catalog Number: G1111). The count of the prostate, lung, and endometrial cells was adjusted to 5×10^4 cells per well using mediums.

Statistical analysis

All experiments were carried out in triplicate (n = 3). Data were presented as mean ± standard deviation (SD). The statistical analysis was performed with statistical software (NCSS). Statistical significance was accepted at $p < 0.05$.

Results and discussion

Total phenolic and flavonoid contents

The quantities of total phenolic and flavonoid compounds of *A. kurtzianum* methanolic extracts are shown in Table 2. It was found that total phenolic and flavonoid compounds of aerial parts were higher than that of the bulbs. Similarly, previous studies reported that aerial parts of other *Allium* species had higher total phenolic and total flavonoid contents than that of the bulbs (Simin *et al.,* 2013; Ceylan and Alıc, 2015).

Phenolic profile by LC-MS/MS

Previous phytochemical studies on *Allium* species have revealed the occurrence of various phenolics and flavonols in aerial parts and bulbs (Putnik *et al.,* 2019). In the present work, among the 60 phenolic compounds investigated by LC-MS/MS analysis, 20 of them were determined in both aerial parts and bulbs, as presented in Table 1. The following compounds were not found in both extracts: (-)-catechin gallate, (-)-epicatechin gallate, (+)-trans taxifolin, 3-O-methyl quercetin, apigenin, caffeic acid phenethyl ester, chicoric, chlorogenic acid, curcumin, dihydrocapsaicin, dihydrokaempferol, ellagic acid, emodin, eupatilin, genistein, glycyrrhizic acid, gypsogenin, hederagenin, hesperidin, isosakuranetin, kaempferol, kaempferol-3-O-glucoside, luteolin, myricetin, myricitrin, naringin, oleanoic acid, oleuropein, orientin, quillaic acid, penduletin, rosmarinic acid, salicylic acid, scutellarein, shatavarin, sinensetin, trans-4-hydroxycinnamic acid, trans-ferulic acid, trans-cinnamic acid, and verbascoside.

Table 2. Total phenolic and flavonoid contents, antioxidant, and cytotoxic activities of methanolic extracts of *A. kurtzianum*.

	Phenolic content (mg GAE/g)	Flavonoid content (mg CE/g)	Antioxidant activity		Cytotoxic activity		
			DPPH (EC_{50}) (mg/mL)	FRAP (mM Fe^{+2})	IC_{50} (mg/mL)		
					PC-3	A549	ECC-1
Aerial part	48.27 ± 3.24[a]	37.34 ± 2.04[a]	4.63 ± 0.13[a]	3.39 ± 0.09[a]	0.096 ± 0.001	0.059 ± 0.006	0.182 ± 0.045
Bulb	21.28 ± 1.96[b]	10.30 ± 1.74[b]	12.42 ± 0.09[b]	1.77 ± 0.07[b]	-	-	-
Quercetin			0.09 ± 0.001[c]	4.19 ± 0.23[c]			
α-tocopherol			0.20 ± 0.003[d]	1.53 ± 0.05[d]			

Values are means ± SD of three replicates (n = 3). Different superscript letters in the same column indicate significant difference ($p < 0.05$). Total phenolic contents are defined as mg gallic acid equivalents (GAE) per g extract; Total flavonoid contents are defined as mg catechin equivalents (CE) per g extract. EC_{50} is the effective concentration which is required to scavenge 50% of DPPH radical. The EC_{50} values were calculated by linear regression analysis from dose-response curves. The reducing power of the extracts was expressed as FRAP value (mM Fe^{+2} equivalents). FRAP values; for extracts at 10 mg/mL and for standards at 0.25 mg/mL concentration. IC_{50} is the extract concentration that is required to inhibit 50% of cell growth. The IC_{50} values were calculated by linear regression analysis from dose-response curves after 48 h of incubation.

The obtained results indicate that aerial parts had richer sources of phenolic constituents as compared to the bulbs. Rutin (32.46 mg/g extract) and hyperoside (13.33 mg/g extract) were the most abundant phenolic compound identified in the aerial parts. Also, considerable amounts of fumaric acid were identified in both aerial parts (4.69 mg/g extract) and bulbs extracts (5.92 mg/g extract). Similarly, the studies on the chemical profiles of the *Allium* species reported that aerial parts are richer in phenolic acids (*p*-coumaric acid, quinic acid, catechin, apigenin, and caffeic acid) than that of the bulbs (Simin *et al.,* 2013; Ceylan and Alıc, 2015). On the other hand, Martins *et al.* (2016) reported that bioactive substances in the bulbs of *A. sativum* are volatile compounds such as ajoen, sulphur-containing compounds such as alliin, allicin, and allixin, and sulphides such as di-, tri-, and tetra-sulphides. Dziri *et al.* (2012) have identified luteoline, apigenine, and kaempferol derivates in the leaves, flowers, stalks, and bulbs extracts of *A. roseum* by HPLC-PDA-MS analysis. In another study, polyphenolic contents of six bulbs of *A. sativum* varieties had been investigated, and various amounts of rutin, caffeic acid, hyperoside, epicatechin, apigenin, quercetin, naringenin, and ferulic acid have been reported (Fratianni *et al.,* 2016). Mollica *et al.* (2018) have analysed bioactive compounds of methanolic extracts from *A. scorodoprasum*, and considerable amounts of rosmarinic acid, quercetin, and rutin have been identified in the bulbs, flowers, and stem extracts. It can be seen that the extracts of the other *Allium* species were rich in terms of phenolics, and our results are in agreement with the literature.

Antioxidant activity

In the present work, DPPH and FRAP assays were performed to evaluate the antioxidant potentials of *A. kurtzianum*. DPPH is commonly used to evaluate the ability of an antioxidant to scavenge free radicals (Lee *et al.,* 2019). The results showed that *A. kurtzianum* extracts had DPPH radical scavenging activity in a concentration-dependent manner (Figure 1), and the aerial parts were more active than the bulbs (Table 2). Similar results were also reported for the free radical scavenging activity of different *Allium* species (*A. orientale* and *A. roseum*) (Rouis-Soussi *et al.,* 2014; Ceylan and Alıc, 2015). In FRAP method, the colourless oxidised Fe^{3+} form is converted to a blue-coloured Fe^{2+} TPTZ-reduced form by the action of the electron donation from antioxidants (Gupta *et al.,* 2019). The high reducing properties of many *Allium* species have been demonstrated using FRAP method (Lu *et al.,* 2011). Among the extracts studied, the aerial parts showed high reducing activity (Table 2).

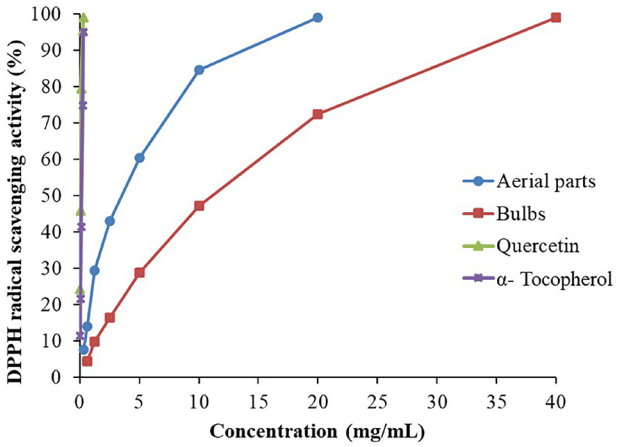

Figure 1. DPPH radical scavenging activity of the methanolic extracts of *A. kurtzianum*.

Also, it was found that there was a strong correlation between the antioxidant activity (DPPH and FRAP value) and the phenolic compounds (*r* value of -0.984 and 0.993, respectively). The antioxidant properties of the *Allium* species have mostly been attributed to their phenolics and organosulfur compounds (Fredotović *et al.,* 2017). In previous studies, it showed that rutin and hyperoside had high DPPH radical scavenging activity, and also, the phenolic compounds were ordered as vitamin C > quercitrin > rutin > hyperoside > catechin > quercetin in terms of IC_{50} values (Zhu *et al.,* 2017). The high antioxidant activity of the aerial parts of *A. kurtzianum* may be due to their flavonols such as rutin and hyperoside.

Antidiabetic activity

Diabetes mellitus is a chronic endocrine disease that affects carbohydrate metabolism. Inhibitors of carbohydrate digestive enzymes (α-glucosidase and α-amylase) were used as oral hypoglycaemic drugs for the control of blood glucose in diabetes mellitus (Nickavar and Yousefian, 2009). The extracts from aerial parts and bulbs of *A. kurtzianum* showed no valuable inhibition on α-glucosidase and α-amylase. On the contrary, the inhibitory effects of six *Allium* species on α-amylase activity were reported by Nickavar and Yousefian (2009). However, in that study, none of the extracts exhibited inhibition as efficiently as acarbose.

DNA protection activity

The interaction of the reactive oxygen species with DNA results in the generation of

damaged bases or strand breaks (Valko *et al.,* 2004). This fact is biologically significant as damage to the genome has been attributed to the development of several cancers and many diseases. The hydroxyl radical (•OH) is the most reactive oxygen-derived radical, and can oxidise DNA. In DNA nicking assay, the native supercoiled plasmid DNA transforms into linear and nicked circular forms by •OH (Leba *et al.,* 2014).

The results obtained showed that both extracts dose-dependently protected the DNA against the harmful effects of •OH (Figures 2 and 3). As compared to the negative control (DNA + Fenton's Reagent), the most effective protection (92.05%) of DNA was obtained with the extract of aerial parts at 50 µg/mL. Figure 3 also shows that the nicked DNA formation was only 43.69% (aerial parts) and 56.42% (bulbs), at the lowest dose of the extracts (1.56 µg/mL). Only two studies showed the DNA protective effects of different *Allium* species (*A. cepa* and *A. x cornutum*) using DNA nicking assay (Prakash *et al.,* 2007; Fredotović *et al.,* 2017). The phenolic compounds are potential protective agents against oxidative stress, and offer protection of DNA by chelating transition metal ions (Prakash *et al.,* 2007). Previous studies showed that hyperoside, rutin, and quercitrin have high DNA protective effects (Zhu *et al.,* 2017). The phenolic compounds present in extracts of the *A. kurtzianum* (especially hyperoside and rutin) might also be responsible for their DNA protection capacities.

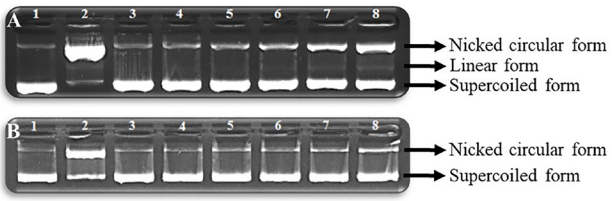

Figure 2. The DNA protective effect of aerial parts (A) and bulbs (B) of *A. kurtzianum* extracts. Lane 1: DNA + distilled water (control); Lane 2 DNA + Fenton's Reagent-FR (negative control); Lane 3: DNA + FR treated with extract (50 µg/mL); Lane 4: DNA + FR treated with extract (25 µg/mL); Lane 5: DNA + FR treated with extract (12.5 µg/mL); Lane 6: DNA + FR treated with extract (6.25 µg/mL); Lane 7: DNA + FR treated with extract (3.13 µg/mL); and Lane 8: DNA + FR treated with extract (1.56 µg/mL).

Cytotoxic activity

Epidemiological studies have found that increased consumption of *Allium* species reduces the risk of some cancers such as prostate, stomach, endometrial, and colon (Tepe *et al.,* 2005; Upadhyay, 2016). The cytotoxic activity of *A. kurtzianum* is shown in Table 2. The aerial parts exhibited cytotoxic activity against prostate (PC-3), lung (A549), and endometrium (ECC-1) cancer cell lines. On the contrary, the bulbs did not show any cytotoxic effect. The aerial parts showed the highest cytotoxicity on A549 cell line. The cytotoxic effects of the aerial parts may be related to its high amount of rutin. *In vivo* studies showed that quercetin has an antitumor effect in mice. Also, a similar effect has been reported by rutin (the glycoside of quercetin) (Sengupta *et al.,* 2004). *In vitro* cytotoxic activities of various *Allium* species on cancer cells such as HepG2, Caco-2, A-1235, MCF-7, and MDA-MB-231 have been reported in many studies (Fredotović *et al.,* 2017; Abdel-Hady *et al.,* 2018; Vafaee *et al.,* 2019). It was suggested that the cytotoxic effects of *Allium* species may be related to their organosulfur constituents (Tepe *et al.,* 2005). Kim *et al.* (2008) showed that thiosulfinates from *A. tuberosum* inhibited PC-3 cell proliferation. There have been several studies revealing the preventive effects of organosulfur compounds on cancer, cell cycle progression, and angiogenesis (Beit-Yannai *et al.,* 2011; Yu *et al.,* 2015; Abdelrahman *et al.,* 2017).

Conclusion

The present work describes for the first time

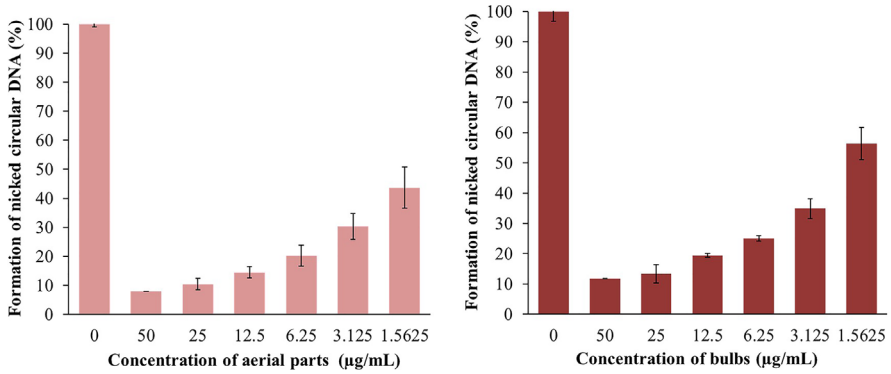

Figure 3. The effect of the extracts on the formation of nicked circular DNA.

the phenolic profile and antioxidant, antidiabetic, DNA protective, and anticancer effects of endemic *A. kurtzianum*. The results demonstrated that the methanolic extracts have antioxidant, anticancer, and DNA protection activities. Also, the chromatographic analysis results showed that the aerial parts are rich in phenolics, such as rutin, hyperoside, and fumaric acid. The phenolic compounds present in the extract might be responsible for the above mentioned activities of *A. kurtzianum*. The results of the present work suggest that, *A. kurtzianum* might be an important source for the development of new antioxidant, DNA protective, and chemopreventive agents.

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