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Original article

Evaluation of cytotoxic and apoptotic effects of the extracts and phenolic compounds of *Astragalus globosus* Vahl and *Astragalus breviflorus* DC

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ABSTRACT

Astragalus L. is a genus member of the Fabaceae family, representing about 3,000 species all over the world and 380 species in Turkey. *Astragalus* species have been used in traditional medicine for many years. *Astragalus globosus* Vahl, known as "top geven", is a dwarf, scapose, perennial herb, *Astragalus breviflorus* DC., known as "yünlü geven", is an extremely spiny dwarf shrub. These endemic species grow in the Turkish cities of Erzurum, Kars, and Van. This is the first phytochemical and cytotoxic investigation of *Astragalus globosus* Vahl and *Astragalus breviflorus* DC. The main extracts and sub-fractions from the plants were evaluated for *in vitro* cytotoxic and apoptotic activities. The IC_{50} values of dichloromethane, *n*-butanol, and water extracts of the aerial parts of *A. globosus* against the MCF-7 cell line were determined as 28.39, 868.60, and 1753.00 μg/mL. The values for the MDA-MB-231 cell line were 264.00, 620.30, and 1300.50 μg/mL, respectively. From *A. globosus*, the following were isolated: a flavone glycoside, diosmetin-7-*O*-rutinoside (1); and two flavonol glycosides, isorhamnetin-3-*O*-rutinoside (2) and quercetin-3-*O*-galactoside (3). From *A. breviflorus*, two phenolic acids, caffeic acid (4) and chlorogenic acid (5), and a flavan-3-ol, catechin (6), were isolated. Diosmetin-7-*O*-rutinoside was isolated from *Astragalus* species for the first time and showed the highest cytotoxic activities on the MCF-7 and MDA-MB-231 breast cancer cell lines with IC_{50} values of 13.65 and 12.89 μg/mL, respectively. Moreover, we observed that diosmin exerts cytotoxic effects by causing cell necrosis.

1. Introduction

Worldwide, breast cancer represents 11.7% of all new cancer cases and causes 6.9% of deaths (Sung et al., 2021). Moreover, breast cancer alone constitutes 30% of cancers seen in women. Despite its high incidence, there has been a nearly 40% decrease in breast cancer–related deaths in women for the past thirty years. This mortality decrease can be attributed to the development of new anticancer drugs. Drugs isolated from plants and microorganisms or synthesised after isolation constitute a significant percentage of anticancer agents in cancer treatment. The plant kingdom is a potential resource for developing new anticancer drugs that are more effective and have fewer side effects (Temel, 2015). Among anticancer drugs, cytotoxic drugs are chemicals that damage cells at a certain stage of the cell cycle through mechanisms such as inhibiting certain cellular functions and, thus, cell division or the induction of apoptotic or necrotic cell death (Dikmen et al., 2010).

Astragalus L. is a genus member of the Fabaceae family, representing about 3,000 species all over the world and 380 species in Turkey. *Astragalus* species have been used in traditional medicine for many years as antihypertensives, diuretics, choleretics, antimicrobials, and antivirals (Bedir et al., 2000; Lysiuk and Darmohray, 2016). *Astragalus* species have saponins, flavonoids, phenylpropanoids, alkaloids, steroids, and polysaccharides and show

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anti-inflammatory, immunoregulatory, anti-tumour, antioxidative, antidiabetic, antiviral, and hepatoprotective activities (Li et al., 2014; Yang et al., 2013). In the literature, many studies have shown that *Astragalus* species and their active compounds exhibit cytotoxic effects on colon carcinoma (HCT-116, HT-29), hepatocellular carcinoma (HEPG2), myeloid leukaemia (K 562), lymphocytic leukaemia (SKW-3), osteosarcoma (MG63), and breast cancer (MCF-7, MDA-MB-231) cell lines (Ibrahim et al., 2013; Horo et al., 2016; Salem et al., 2020; Ionkova et al., 2010). *Astragalus globosus* Vahl (syn. *Astragalus cylindraceus* DC), known as "top geven" in Turkey, grows in the Turkish cities of Erzurum, Kars, Erzincan, and Van. *Astragalus breviflorus*, known as "yünlü geven," grows in Erzurum, Muş, and Van, Turkey (Chamberlain and Matthews, 1970; Güner et al., 2012). The antimutagenic and antioxidant activities of *Astragalus globosus* have been studied (Özbek et al., 2009; Güllüce et al., 2008), but no previous studies have been published on the phytochemical and cytotoxic activity of the *A. globosus* and *A. breviflorus* endemic species in our knowledge.

This study aimed to determine the *in vitro* cytotoxic and apoptotic effects of the extracts and phenolic compounds from *Astragalus globosus* Vahl and *Astragalus breviflorus* DC.

2. Materials and methods

2.1. General experimental procedures

Silica gel 60 (0.063–0.200 mm, Merck), Polyamide 6 (Sigma–Aldrich), Lichroprep RP-18 (25–40 μm, Merck), and Sephadex LH-20 (Fluka) were used to perform column chromatography, while pre-coated Kieselgel 60 F254 aluminium sheets (Merck) were used for thin layer chromatography. The isolated compounds were determined using UV fluorescence and 1% vanillin-H_2SO_4 reagent. ^1H and ^{13}C NMR spectra were recorded on a Varian Mercury Plus 400 MHz for proton and 100 MHz for carbon NMR, with TMS being the internal standard. The solvents used were DMSO d_6 and CD_3OD. HR-ESI-MS was performed on an Agilent 6530 Accurate-Mass. HPLC was applied using an Agilent Technologies 1260 Infinity with DAD detector and Supelco–Ascentis RP Amide Column (25 cm × 10 mm, 5 μm).

2.2. Plant material

Astragalus globosus and *A. breviflorus* were collected in Erzurum, Turkey, and authenticated by Mehmet Önal. The herbarium specimens of the plants (AUEF 1375 and AUEF 1376) were deposited at the Biodiversity Application and Research Center of Atatürk University, Erzurum, Turkey.

2.3. Extraction and isolation

Air-dried and powdered aerial parts (363.133 g) and roots (139.747 g) from *Astragalus globosus* were extracted three times with 80% ethanol at 40 °C (3 × 4 L) in different balloons. Ethanol was evaporated to dryness after filtration, and 97.726 g (26.9%) of ethanol extract from the aerial parts and 26.008 g (18.61%) of ethanol extract from the roots were obtained. The extracts were dissolved in water and partitioned using dichloromethane (8 × 0.4 L) and *n*-butanol (12 × 0.4 L). Furthermore, 16.772 g (4.61%) of dichloromethane, 64.989 g (17.89%) of *n*-butanol, and 15.742 g (4.33%) of aqueous extracts were obtained from the aerial parts; 4.771 g (3.41%) of dichloromethane, 17.390 g (12.44%) of *n*-butanol, and 3.979 g (2.84%) of aqueous extracts were obtained from the roots.

The *n*-Butanol extract from the aerial parts of *A. globosus* was separated via polyamide column with H_2O: MeOH mixtures (100: 0 → 0:100) to produce Fraction (Fr.) A (22.511 g), Fr. B (1.471 g), Fr. C (1.552 g), and Fr. D (899.8 mg). Fraction B was precipitated using methanol. The resulting precipitate was concentrated to dryness, and **Compound 1** (10.7 mg) was obtained. After precipitation, the remainder of Fraction B was exerted to a Sephadex LH-20 column and eluted with MeOH to afford Fr. B_1 and Fr. B_2. Fr. B_2 was subjected by reversed-phase column chromatography using MeOH: H_2O (0:100 → 100:0) to produce Fr. $B_{2.1}$ and Fr. $B_{2.2}$. Fr. $B_{2.2}$ was separated via semi-preparative HPLC by a gradient solvent system of MeOH:0.2% formic acid in H_2O (40:60 → 60:40) to produce **Compound 2** (17.3 mg, t_R:39.8 min). Fr. D was separated on a reversed-phase silica gel column with MeOH: H_2O (0:100 → 100 :0) to yield Fr. D_1, Fr. D_2, and Fr. D_3. Fr. D_2 was separated using semi-preparative HPLC with a gradient solvent system of MeOH:0.2% formic acid in H_2O (10:90 → 60:40) to produce **Compound 3** (20 mg, t_R:9.8 min.).

Air-dried and powdered roots of *Astragalus breviflorus* (481.243 g) were extracted three times with 80% ethanol at 40 °C (3 × 4 L). Ethanol was evaporated to dryness, and 94.018 g (19.53%) of ethanol extract was obtained after filtration. The main extract was dissolved in distilled water and partitioned by dichloromethane (7 × 0.4 L) and *n*-butanol (10 × 0.4 L). Finally, 14.998 g (3.11%) of dichloromethane, 66.816 g (13.88%) of *n*-butanol extracts, and 11.867 g (2.46%) of the aqueous fraction were obtained.

The *n*-butanol extract from the roots of *A. breviflorus* was obtained using a silica gel column and eluted with a chloroform: methanol mixture (100:0 → 0:100) to produce Fr. A (372.1 mg), Fr. B (4.409 g), Fr. C (5.419 g), and Fr. D (7.561 g). Fr. B was separated via semi-preparative HPLC using a CH_3CN:H_2O (10:90 → 30:70) gradient solvent system to yield **Compound 4** (20 mg, t_R:7.2 min.). Fr. C was separated via semi-preparative HPLC using a CH_3CN:H_2O (10:90 → 30:70) gradient solvent system, yielding **Compound 5** (19.2 mg, t_R:10.3 min.), and Fr. D was separated via semi-preparative HPLC using a CH_3CN:H_2O (10:90 → 30:70) gradient solvent system to obtain **Compound 6** (9.2 mg, t_R:7.0 min.).

2.4. In vitro cytotoxicity assay

MTT assay was preferred to evaluate the cytotoxic activities of the extracts and isolated compounds from *A. globosus* and *A. breviflorus*. MCF-10A, MCF-7, and MDA-MB-231 cell lines were taken from the American Type Culture Collection. Breast cancer cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Sigma-Aldrich) with 10% fetal bovine serum (FBS) (Sigma-Aldrich), while non-tumourigenic breast epithelial cells were cultured in DMEM-F12 medium (Sigma-Aldrich) with 10% FBS (Sigma-Aldrich). The humidified 5% CO_2 incubator was used to incubate the cultures at 37 °C. The cells (1×10^4 cells/well) were seeded in 96-well plates. Different concentrations of extracts and compounds were treated. Phosphate-buffered saline (PBS) was used to wash the extracts after 24 h. To each well, 20 μL of the MTT solution was added, and the plate was incubated for 4 h at 37 °C; 100 μL of DMSO was added to dissolve the formazan crystals. Absorbance values were measured at 570 nm by an ELISA reader (Epoch). Measurements were repeated three times. The percentage of cell viability and IC_{50} values were calculated. IC_{50} values were calculated using GraphPad 6.0 software.

2.5. Flow cytometry

Cell death types were determined using the Annexin V-FITC/PI (Biolegend, 640914) via flow cytometry of the cell lines. The cell lines (3×10^5 cells/2 mL per well) were seeded in six-well plates and then incubated for 24 h at 37 °C in 5% CO_2. The cell lines were treated with the highest doses of Compounds 1–6 for 48 h. The

cells were harvested from the surfaces with trypsin after incubation. PBS was used to wash the cells. Then, the cells were centrifugated at 1500 rpm for five minutes, and the cell pellets were resuspended in 1 X binding buffer. Five microlitres of fluorochrome-conjugated Annexin V and 10 μL of PI were treated with 100 μL of cell suspension and then incubated for 15 min at 37 °C in the dark. Then, 400 μL of 1 X binding buffer was added. Each sample was analysed via flow cytometry (Beckman Coulter CytoFLEX Flow Cytometer), and the data were obtained using CytExpert software. The amounts of viable, early–late apoptotic, and necrotic cells were given as a percentage of the total population. Measurements were repeated three times.

2.6. Statistical analysis

Cell viability differences between groups were evaluated via the one-way ANOVA–Dunnett's test using GraphPad 6.0 software, and $p < 0.05$ was considered significant.

3. Results

3.1. Isolation and characterisation

After isolation from *A. globosus*, three yellow amorphous powder compounds were obtained—a flavone glycoside, diosmin (1); and two flavonol glycosides, narcissin (2) and hyperoside (3). Furthermore, two phenolic acids, caffeic acid (4) and chlorogenic acid (5), and a flavan-3-ol, catechin (6), were isolated from *A. breviflorus* as a white amorphous powder. The main structures of the compounds are shown in Fig. 1.

Diosmin (Diosmetin-7-*O*-rutinoside) (1): HR-ESI-MS (*m*/*z*) 609.1814 $[M + H]^+$ (calculated for $C_{28}H_{32}O_{15}$, 608.5355).

Narcissin (Isorhamnetin-3-*O*-rutinoside) (2): HR-ESI-MS (*m*/*z*) 625.1765 $[M + H]^+$ (calculated for $C_{28}H_{32}O_{16}$, 624.5345).

Hyperoside (Quercetin-3-*O*-galactoside) (3): HR-ESI-MS (*m*/*z*) 465.1015 $[M + H]^+$ (calculated for $C_{21}H_{20}O_{12}$, 464.3695).

Caffeic acid (4): HR-ESI-MS (*m*/*z*) 181.0476 $[M + H]^+$ (calculated for $C_9H_9O_4$, 181.0295).

Chlorogenic acid (5): HR-ESI-MS (*m*/*z*) 355.1012 $[M + H]^+$ (calculated for $C_{16}H_{18}O_9$, 354.3033).

Catechin (6): HR-ESI-MS (*m*/*z*) 291.0852 $[M + H]^+$ (calculated for $C_{15}H_{14}O_6$, 290.2643).

3.2. Cytotoxicity evaluation

All the extracts and compounds were tested for their cytotoxic activities on a non-tumorigenic breast epithelial cell line (MCF-10A) and breast cancer cell lines (MCF-7 and MDA-MB-231) using MTT assay with hydrogen peroxide as the positive control. It was demonstrated that a variety of *in vitro* cultured tumour cells, when treated with hydrogen peroxide, are inactivated rapidly (Symons et al., 2001). Hydrogen peroxide was used as the positive control in this study because of its rapid effects, and it is relatively cheap and cost-effective.

Firstly, prestudies for all extracts of the aerial parts and roots of *Astragalus globosus* and *Astragalus breviflorus* were carried out to determine their *in vitro* cytotoxic effects on the breast cancer cell lines. As a result of preliminary experiments, dichloromethane, *n*-butanol, and water extracts of aerial parts of *Astragalus breviflorus* were found to be ineffective. Thus, it was decided to carry out further studies on other extracts.

The dichloromethane extracts from the aerial parts and roots of *A. globosus* were more effective on the breast cancer cell lines than the *n*-butanol and water extracts (0.38, 1.5, 6.0 mg/mL). At 24 h, the cell viabilities for the dichloromethane extract from the aerial parts of *A. globosus* (1.5 mg/mL) were determined as 14%, 25%, and 19% on the MCF-10A, MCF-7, and MDA-MB-231 cell lines, respectively. For the dichloromethane extract from the roots of *A. globosus* (1.5 mg/mL), these values were 18%, 21%, and 14%, respectively.

The dichloromethane extract from the roots of *Astragalus breviflorus* was more effective on the breast cancer cell lines than the *n*-butanol and water extracts (0.38, 1.5, 6.0 mg/mL). At 24 h, the cell viabilities for the dichloromethane extract from the roots of *A. breviflorus* (1.5 mg/mL) were determined to be 69%, 52%, and 59% on the MCF-10A, MDA-MB-231, and MCF-7 cell lines, respectively. For the *n*-butanol extract from the roots of *A. breviflorus* (1.5 mg/mL), the values were 79%, 38%, and 66%, respectively.

The dichloromethane extract of the aerial parts of *A. globosus* (IC_{50}: 28.39 μg/mL) had the best cytotoxic activity on the MCF-7 cell line. The dichloromethane extract of the roots of *A. globosus* had the best cytotoxic activity on the MDA-MB-231 cell line (IC_{50}: 82.49 μg/mL). The IC_{50} values of dichloromethane and *n*-butanol extracts of the aerial parts of *A. globosus* on the MCF-10A cell line were determined as 85.42 and 1471.00 μg/mL, respectively. *n*-Butanol extracts of *A. globosus* had lower cytotoxic effects on the MCF-10A cell line. The most effective sub-extract of the roots of *A. breviflorus* was dichloromethane extract on the MCF-7 cell line. *n*-butanol extract of *A. breviflorus* was the most effective on the MDA-MB-231 cell line. Among these two extracts, *n*-butanol extract had lower cytotoxic effects on healthy breast epithelial cell line (IC_{50}: 1984.00 μg/mL).

The cell morphology was examined under an inverted light microscope. At 24 h, 0.75 mg/mL of *n*-butanol extracts and 0.09 mg/mL of dichloromethane extracts caused morphological changes on MCF-10A cell line.

It was found that *n*-butanol extracts contain phenolic compounds by thin layer chromatography analysis. Because of its low cytotoxic effects on healthy cells and rich phenolic content, it was decided to perform isolation studies on the *n*-butanol extracts.

The compounds showed dose-dependent cytotoxic effects on the cancer cell lines. Compound 1 exhibited the best cytotoxic effects on the MCF-7 and MDA-MB-231 cell lines with IC_{50} values of 13.65 ± 11.04 and 12.89 ± 12.25 μg/mL, respectively. The IC_{50} values of Compound 2 were 137.10 ± 8.62 and 77.78 ± 39.88 μg/mL on the MCF-7 and MDA-MB-231 cell lines, respectively. Compound 3 did not show cytotoxic activity up to 100 μg/mL on the breast cancer cell lines in this study. Compounds 4 and 6 exhibited cytotoxic effects on the MCF-7 and MDA-MB-231 cell lines, with IC_{50} values of 40.32 ± 2.84 and 64.04 ± 4.11 μg/mL and 34.03 ± 19.77 and 31.83 ± 23.41 μg/mL, respectively. Compound 5 did not exhibit cytotoxic activity up to 360 μg/mL against the breast cancer cell lines. The IC_{50} values of the extracts and compounds are presented in Tables 1 and 2.

3.3. Flow cytometry

Apoptosis was detected via Annexin V-FITC/PI by flow cytometry. In the negative control group and the H_2O_2 (17 μg/mL), Compound 1 (24 μg/mL), Compound 2 (156 μg/mL), Compound 3 (372 μg/mL), Compound 4 (30 μg/mL), Compound 5 (710 μg/mL), and Compound 6 (60 μg/mL) groups, the viable cell amounts on the MCF-7 cell line were found to be 83%, 30%, 81%, 71%, 74%, 46%, 57%, and 58%, respectively. The highest cell death was seen by necrosis at 29% for Compound 2. The percentage of cell death was 70% in the group to which H_2O_2 was applied. The highest apoptosis rates were observed for Compound 4 (necrotic cell: 3%, early apoptotic cell: 52%, late apoptotic cell: 13%).

In the negative control group and the H_2O_2, Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, and Compound 6 groups, the viable cell amounts on the MDA-MB-231 cell line were found to be 88%, 21%, 56%, 63%, 46%, 30%, 51%, and 75%,

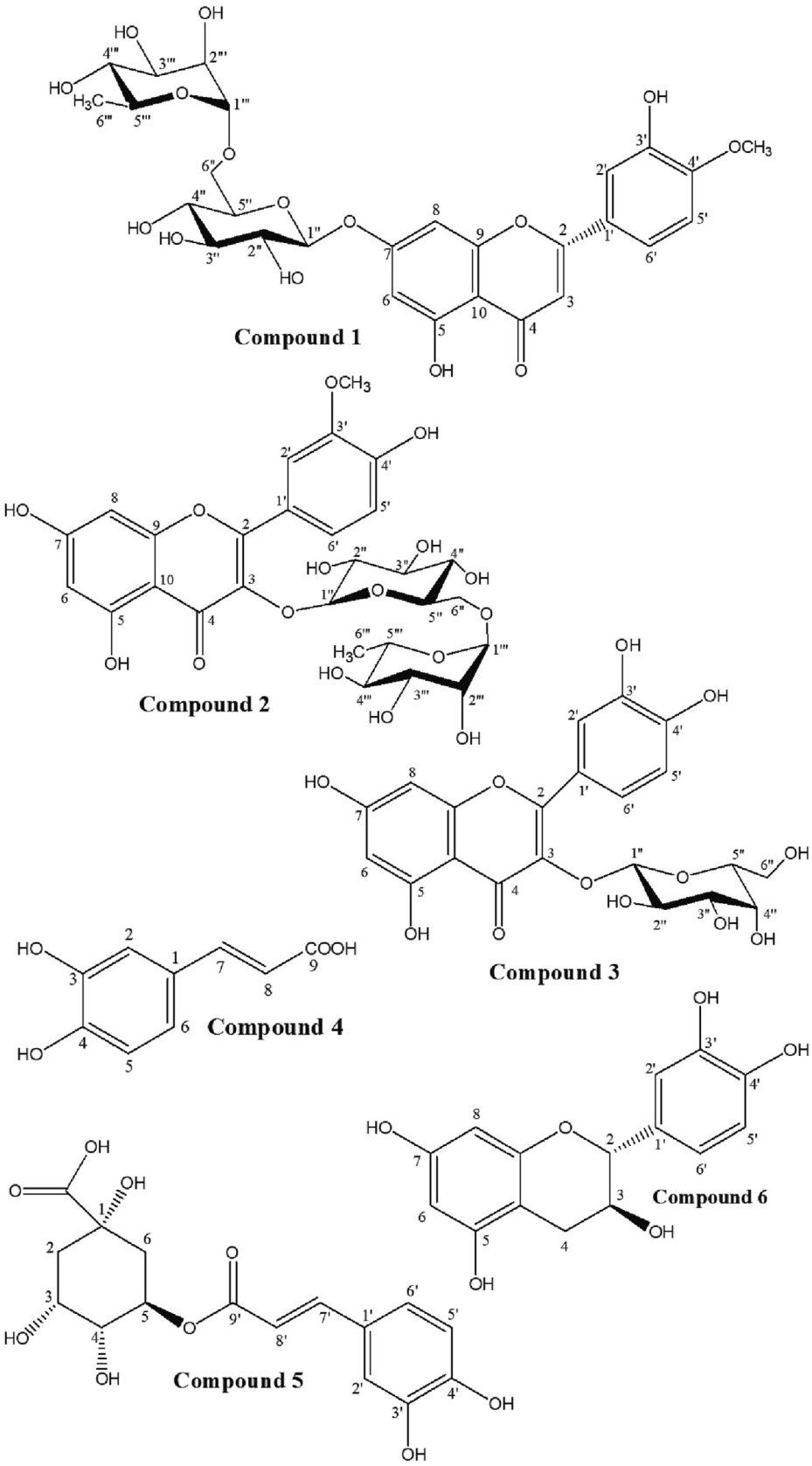

Fig. 1. Structures of Compounds 1–6.

Table 1
IC_{50} values of H_2O_2 and the extracts of *A. globosus* and *A. breviflorus.*

Extracts	Cell Line/IC_{50} (µg/mL)		
Aerial parts of *A. globosus*	**MCF-7**	**MDA-MB-231**	**MCF-10A**
Ethanol	744.90 ± 6.00	879.10 ± 2.34	972.40 ± 2.22
Dichloromethane	28.39 ± 3.01	264.00 ± 8.56	85.42 ± 3.14
n-Butanol	868.60 ± 9.70	620.30 ± 11.48	1471.00 ± 10.85
Water	1753.00 ± 21.57	1300.50 ± 8.49	1049.00 ± 9.72
H_2O_2	11.97 ± 6.72	8.48 ± 3.53	9.77 ± 8.56
Roots of *A. globosus*			
Ethanol	601.00 ± 5.28	354.80 ± 7.09	1090.00 ± 5.13
Dichloromethane	189.20 ± 2.32	82.49 ± 1.25	237.60 ± 7.88
n-Butanol	1151.00 ± 10.94	1703.00 ± 8.48	1352.00 ± 12.68
Water	1217.00 ± 10.67	572.50 ± 6.95	590.00 ± 7.55
H_2O_2	7.45 ± 12.77	8.48 ± 3.53	10.32 ± 3.60
Roots of *A. breviflorus*			
Ethanol	1307.00 ± 6.26	2020.00 ± 4.70	1880.00 ± 11.31
Dichloromethane	1148.00 ± 12.44	1080.00 ± 9.14	1746.00 ± 12.38
n-Butanol	1612.00 ± 12.10	687.50 ± 6.47	1984.00 ± 7.36
Water	1328.00 ± 9.48	1746.00 ± 16.18	469.60 ± 29.54
H_2O_2	10.11 ± 0.90	11.86 ± 2.43	11.40 ± 3.60

IC_{50} values are given as ± standard deviation.

respectively. The highest cell death was observed at 54% in the group treated with Compound 3 (necrotic cell: 6%, early apoptotic cell: 22%, late apoptotic cell: 26%). The percentage of cell death was 79% in the group treated with H_2O_2. The highest cell death was observed at 69% in the group treated with Compound 4 (necrotic cell: 3%, early apoptotic cell: 52%, late apoptotic cell: 13%).

Table 2
IC_{50} values of H_2O_2 and Compounds 1–6.

Compound	Cell Line/IC_{50} (µg/mL)		
	MCF-7	**MDA-MB-231**	**MCF-10A**
1	13.65 ± 11.04	12.89 ± 12.25	8.66 ± 20.51
2	137.10 ± 8.62	77.78 ± 39.88	133.40 ± 23.54
3	147.20 ± 7.32	141.40 ± 13.98	126.9 ± 12.47
4	40.32 ± 2.84	64.04 ± 4.11	148.60 ± 14.07
5	370.80 ± 9.49	361.40 ± 4.83	356.30 ± 19.10
6	34.03 ± 19.77	31.83 ± 23.41	33.97 ± 10.41
H_2O_2	9.91 ± 0.31	11.45 ± 1.52	12.58 ± 9.77

IC_{50} values are given as ± standard deviation.

Figs. 2-7 show apoptosis data for all compounds.

4. Discussion

This is the first phytochemical and cytotoxic investigation of *Astragalus globosus* Vahl and *Astragalus breviflorus* DC. (Fabaceae). The spectroscopic data were compared with the literature to identify the compounds, and all compounds were consistent with the spectral data described in the literature (Numanov et al., 2013; El-Hawiet et al., 2010; He et al., 2010; Forino et al., 2016; Jin et al., 2005; Mrabti et al., 2018). *Astragalus* species attract attention with their anticancer effect potential. *Astragalus* species attract attention with their anticancer effect potential.

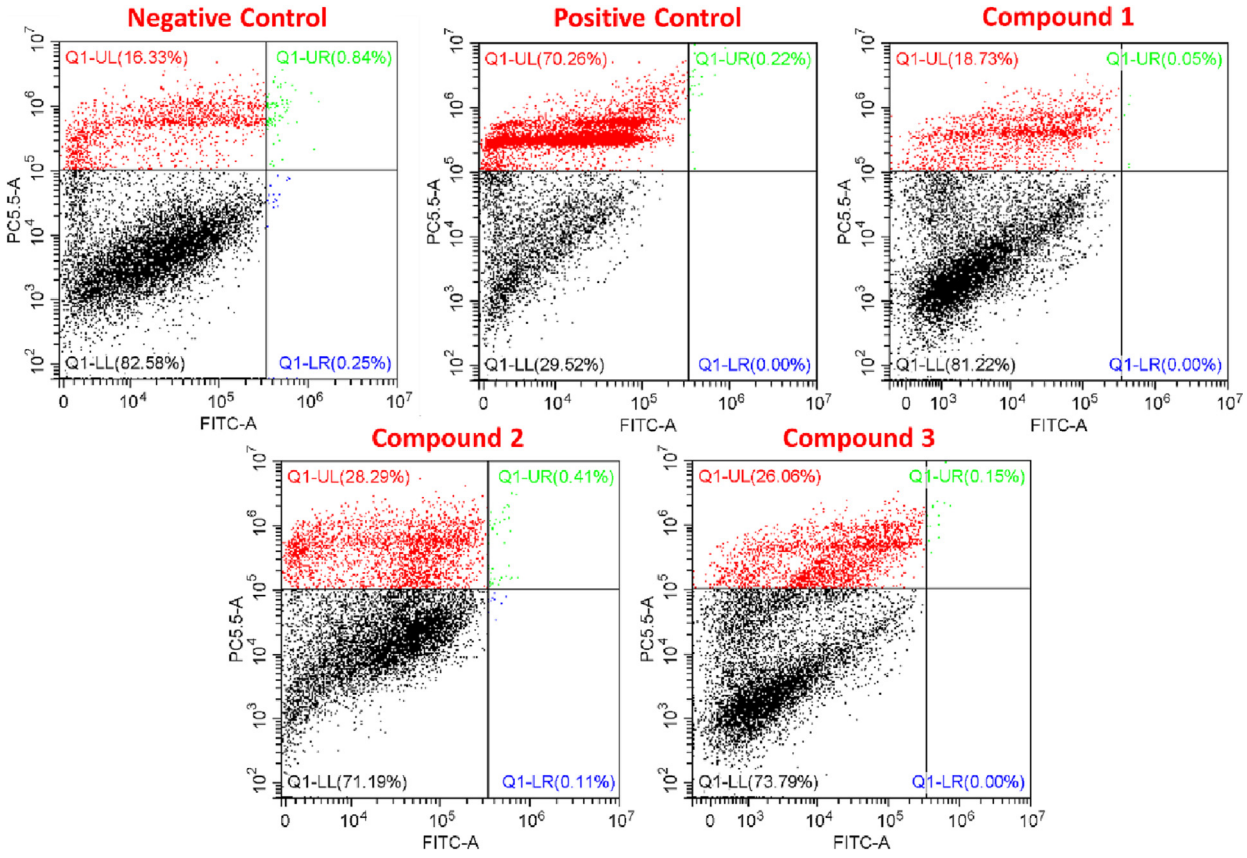

Fig. 2. Viable, early apoptotic, late apoptotic, and necrotic cell populations after application of different concentrations of Compounds 1–3 against to MCF-7 cell line for 48 h.

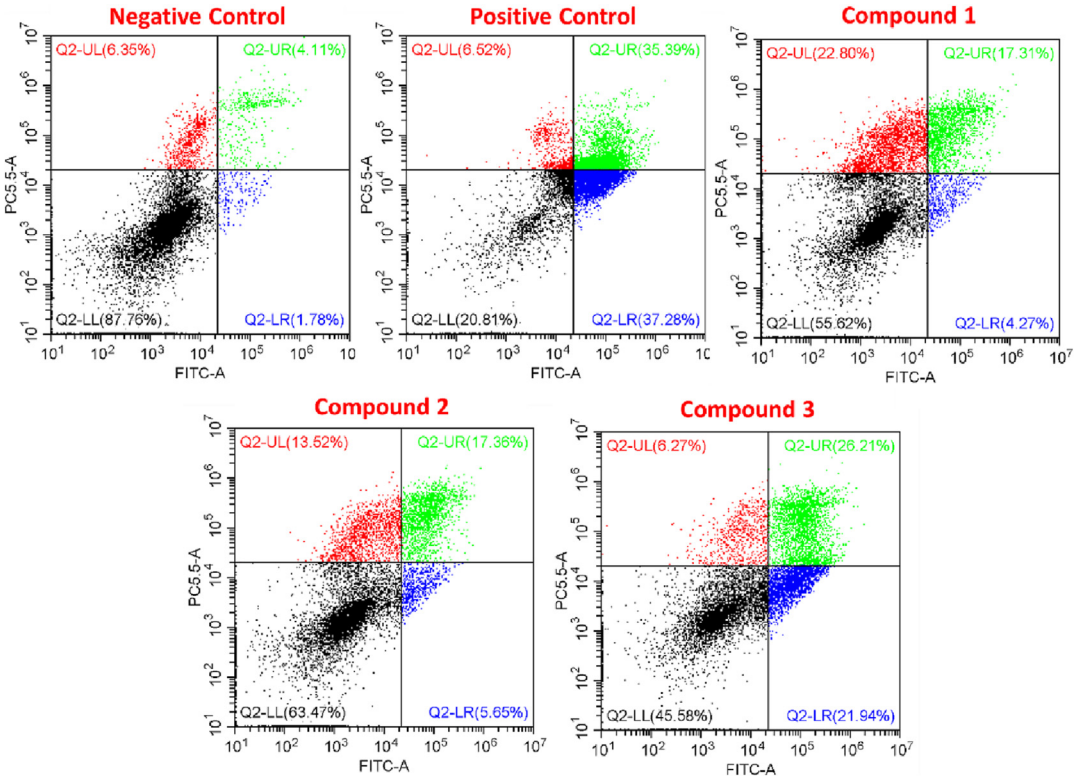

Fig. 3. Viable, early apoptotic, late apoptotic, and necrotic cell populations after application of different concentrations of Compounds 1–3 against to MDA-MB-231 cell line for 48 h.

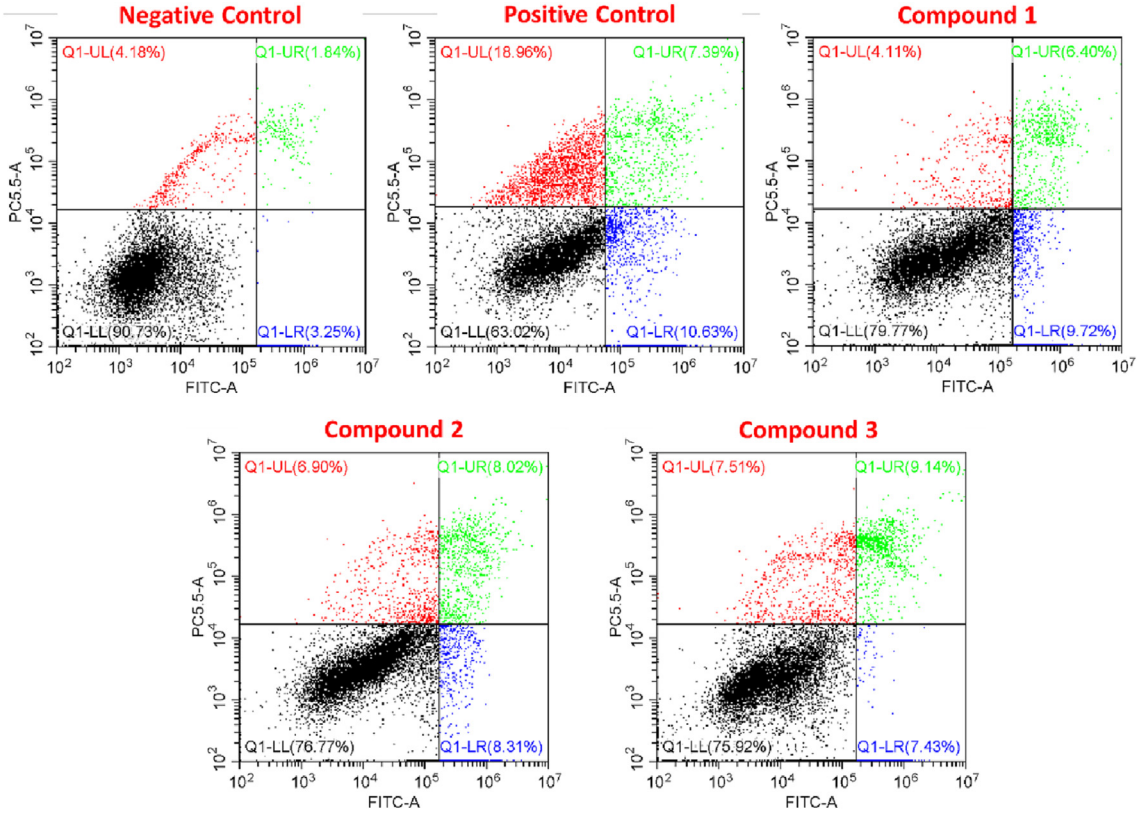

Fig. 4. Viable, early apoptotic, late apoptotic, and necrotic cell populations after application of different concentrations of Compounds 1–3 against to MCF-10A cell line for 48 h.

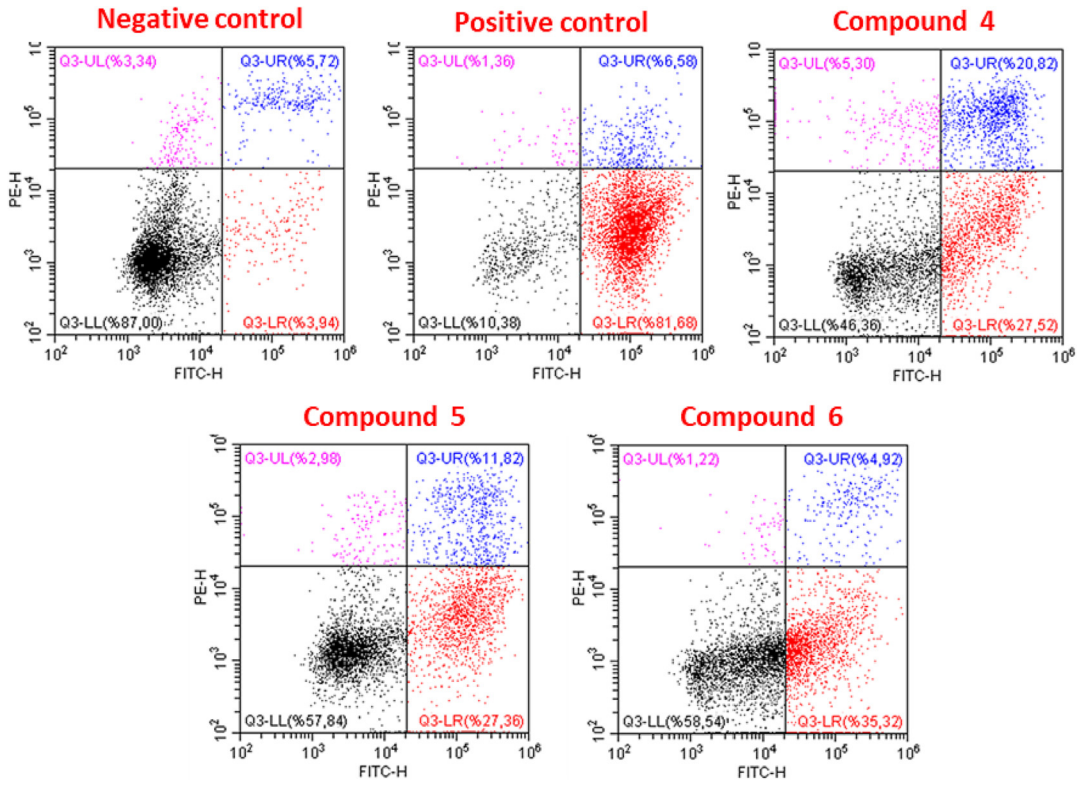

Fig. 5. Viable, early apoptotic, late apoptotic, and necrotic cell populations after application of different concentrations of Compounds 4–6 against to MCF-7 cell line for 48 h.

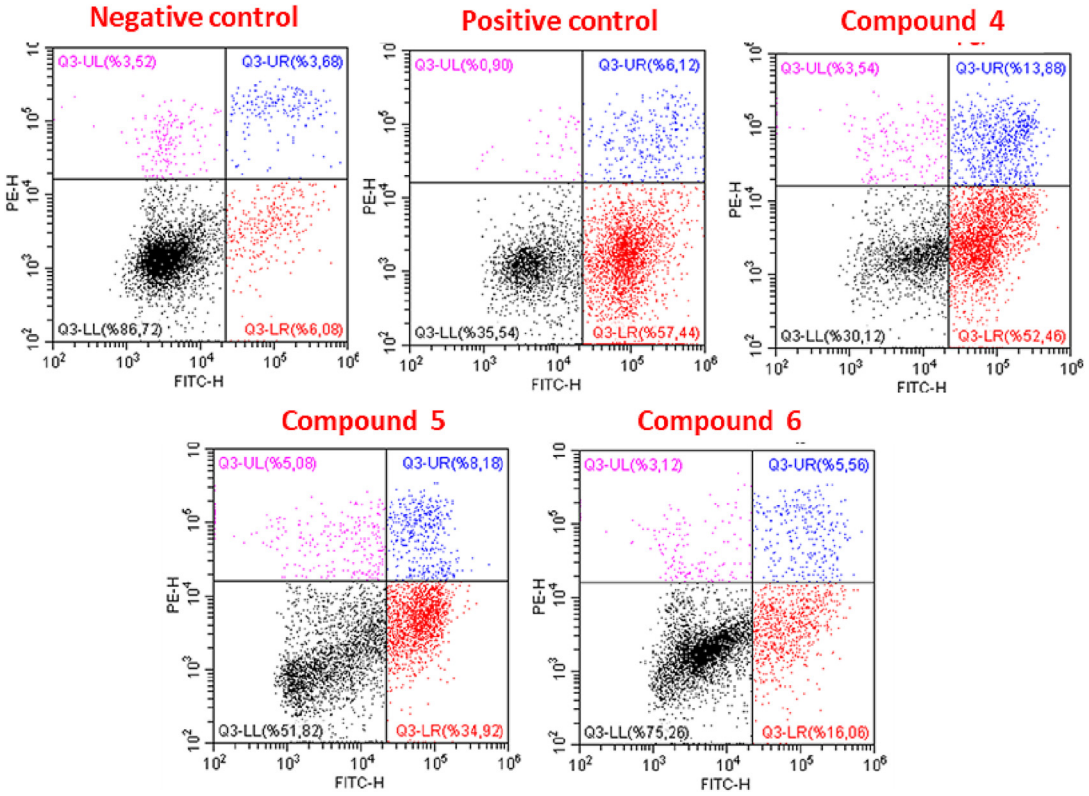

Fig. 6. Viable, early apoptotic, late apoptotic, and necrotic cell populations after application of different concentrations of Compounds 4–6 against to MDA-MB-231 cell line for 48 h.

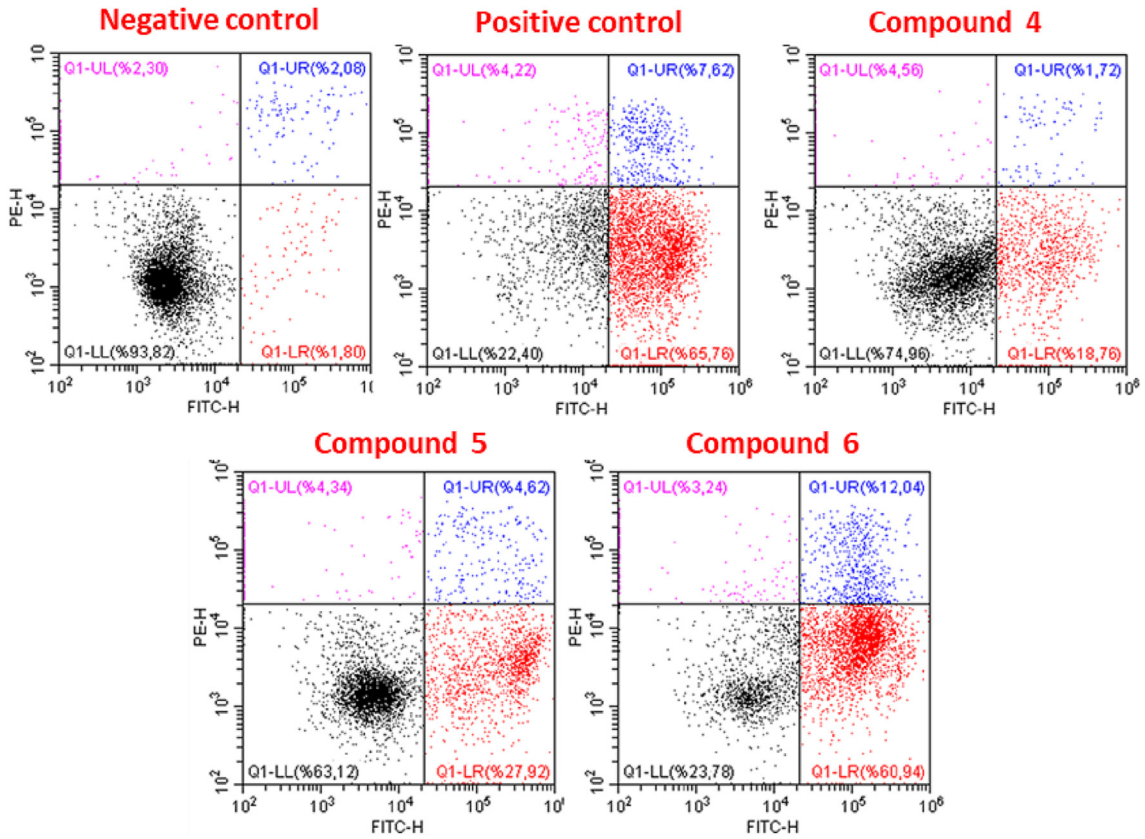

Fig. 7. Viable, early apoptotic, late apoptotic, and necrotic cell populations after application of different concentrations of Compounds 4–6 against to MCF-10A cell line for 48 h.

The medicinal plants are unique resources for developing new anticancer drugs used in the treatment of breast cancer. Many anticancer agents have been isolated from plants, such as *Camptotheca acuminata, Taxus brevifolia, and Catharanthus roseus*. *Astragalus* species attract attention with their anticancer activity potential. In the literature, many studies have found that *Astragalus* species and their active compounds show cytotoxic effects.

In a previous study in which the cytotoxic effects of *Astragalus sieberi* on the MCF-7 cell line was evaluated with an MTT assay, the IC_{50} value of the *A. sieberi* ethyl acetate extract was 69.6 μg/mL (Salem et al., 2020). In another study, the cytotoxic effects of extracts prepared from the aerial parts of *A. bombycinus* on MCF-7 cells were evaluated by MTT assay. Cell viability values for the ethyl acetate, *n*-butanol, methanol, and aqueous extracts of *A. bombycinus* were found to be 56.8%, 68.3%, 23.1%, and 45.3%, respectively (Ibrahim et al., 2013). The dichloromethane extract of *Astragalus globosus* was more effective than *A. sieberi* and *A. bombycinus* extracts on the MCF-7 cell line. The methanolic extracts of *A. danicus* and *A. inopinatus* had low inhibitory effects on the growth of HeLa cells (Gromova et al. 2001). The effects of lectin from the roots of *A. mongholicus* were evaluated on the human cervical carcinoma cell line (HeLa), human leukaemia cell line (K 562), and human osteoblast-like cell line (MG63). Maximum cell growth inhibition was 92%, 84%, and 48%, respectively (Yan et al. 2009). A water-soluble polysaccharide (APS4) isolated from *A. membranaceus* suppressed human gastric carcinoma (MGC-803) cell proliferation by apoptosis-induced and concentration-dependent (Yu et al., 2019). In this study, phenolic compounds of *Astragalus* species have cytotoxic effects apoptosis-induced.

In a previous study, Compound 1 did not demonstrate cytotoxic activity up to 200 μM against DMS-114 human lung, HT-29 colon, DU-145 prostate, and SK-MEL5 melanoma cancer cell lines (Manthey and Guthrie, 2002). In another study, the treatment with 50 μM and 250 μM of Compound 1 decreased DU145 prostate cancer cell viability to 70% and 40%, respectively. Compound 1 lowered prostate cancer cell viability and proliferation (Lewinska et al., 2015). This compound was found to dose-dependently reduce the cell viability of A431 cells by MTT assay, and the IC_{50} value was found to be 45 μg/mL (Buddhan and Manoharan, 2017). Compound 1 exhibited the best cytotoxic effects on the breast cancer cell lines in our study. In a previous study, Compound 2 did not show cytotoxic activity up to 100 μmol/L on the IGROV-1 and OVCAR-3 human ovary cell lines and HCT-116 human colon cancer cell line (Znati et al., 2014). It was also determined that narcissin (25–400 μg/mL) had no significant hepatoprotective activity when tested on HEP-G2 hepatocellular carcinoma cells for 72 h by MTT assay (Gevrenova et al., 2016). In the present study, the IC_{50} values of Compound 2 were 137.10 ± 8.62 and 77.78 ± 39.88 μg/mL on the MCF-7 and MDA-MB-231 cell lines, respectively. However, Compound 3 has demonstrated cytotoxicity at IC_{50} values of 50 μM on MIA PaCa-2 and INS-1 pancreatic cancer cell lines (Boukes and Venter, 2016). 10–100 μM of Compound 3 inhibited cell viability of the A549 human non small cell lung cancer cell line in a time- and dose-dependent manner. For 24 h, there was a significant increase in the percentage of apoptotic cells in a dose-dependent manner (Yang et al., 2017). Compound 3 did not show cytotoxic activity up to 100 μg/mL on the breast cancer cell lines in the present study. In a previous study, Compound 4 did not demonstrate cytotoxic activity up to 100 μg/mL against HeLa human uterine carcinoma, Bel-7402 human hepatocellular carcinoma, A375-S2 human malignant melanoma, and SGC-7901 human gastric cancer cell lines (Sun et al. 2006). The application of 30, 40, and 50 μg/mL of Compound 4 notably inhibited the HT-1080 fibrosarcoma cell line. After the application of 50 μg/mL of Compound 4, 15% of cell

viability of the HT-1080 fibrosarcoma cell line was observed (Rajendra et al., 2011). Compound 4 exhibited cytotoxic effects on MCF-7 and MDA-MB-231 cell lines in the present study. Compound 5 did not exhibit cytotoxic activity up to 360 μg/mL against the breast cancer cell lines in our study. Compound 5 did not have cytotoxic activity in another study with IC_{50} values of 1095, 882.5, and 940 μM on BT-20, SK-BR-3, and MDA-MB-468 human breast cancer cell lines, respectively (Bender and Atalay, 2018). The CC_{50} values of Compound 5 against HSG salivary gland tumour, HSC-2 human oral squamous cell carcinoma, and HGF human gingival fibroblast cell lines were found to be 1.4, 1.3, and 2.3 mM, respectively (Jiang et al., 2000). 100 μM of Compound 5 enhanced Regorafenib-mediated cell growth inhibition and potentiated the apoptotic effect of Regorafenib on PLC/PRF/5 and HepG2 hepatocellular carcinoma cell lines (Refolo et al., 2018). In another study, Compound 6 showed cytotoxic activity against the Caco-2 colorectal adenocarcinoma cell line with an IC_{50} value of 9 μg/mL (Ogunlaja et al., 2018). Treatment with 30 μg/mL and 100 μg/mL of catechin on the T47D human breast cancer cell line caused 44% and 92% of cell growth inhibition, respectively (Deguchi et al., 2002). Compound 6 inhibited A549 human non small cell lung cancer cell proliferation dose-dependently (Sun et al., 2020). Compound 6 exhibited cytotoxic effects on MCF-7 and MDA-MB-231 cell lines in the present study.

5. Conclusion

In conclusion, this is the first phytochemical and cytotoxic investigation of endemic species of *Astragalus globosus* Vahl and *A. breviflorus* DC. Diosmin, narcissin, and hyperoside from the *n*-butanol extract of *A. globosus* aerial parts, as well as caffeic acid, chlorogenic acid, and catechin from the *n*-butanol extract of *A. breviflorus* roots, were isolated. Diosmin was isolated from the *Astragalus* species for the first time in this study. Diosmin showed the highest cytotoxic effects on the breast cancer cell lines. We observed that diosmin exerts its cytotoxic effect by causing necrosis in cells.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2023.06.015.

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