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Ferruginoside D: A Novel Phenylethanoid from *Verbascum leiocarpum*

Samed Şimşek,^{*,[a]} Hüseyin Akşit,^[b] Ali Aydın,^[c] and Ekrem Köksal^[d]

A novel phenylethanoid, ferruginoside D, together with fourteen known compounds were isolated from the *Verbascum leiocarpum* for the first time. Chemical structures of isolated compounds were determined by spectroscopic analysis including HR-ESI-MS and NMR spectra. The antioxidant, α -glucosidase inhibitory properties, and antiproliferative activities against multiple cell lines (A172, C6, HeLa, A2780, SW620, HT29, Beas2B, RPE, and HSF) of the isolated compounds were evaluated *in vitro*. According to the results, iridoids, flavonoids (luteolin and apigenin), and phenylethanoids (poliumoside and isover-

bascoside) with strong antiproliferative potential were also found to have cytostatic effects. Furthermore, the investigation revealed that compounds luteolin, poliumoside, verbascoside, isoverbascoside, ferruginoside C, ferruginoside D, and ursolic acid show potent α -glucosidase inhibitory activity, while compounds luteolin, verbascoside, and isoverbascoside exhibit substantial antioxidant activity. The new compound (ferruginoside D) was found moderately active against cancer cell lines, with strong α -glucosidase inhibitory activity, and moderate antioxidant properties.

Introduction

Natural products of herbal origin constitute a source of many bioactive substances used for therapeutic purposes.^[1] Knowing the medicinal properties of the plants on earth is of great importance due to their contributions to human health and the economy of the countries.^[2] Studies reporting the biological activities of the plant extracts serve as a reference for the discovery of plants with therapeutic potential for many diseases.^[3] Türkiye is the host of many different kinds of plants because of having different geographical regions.^[4] There are more than ten thousand plant taxa in Türkiye and about four thousand of them are endemic.^[5] Species of the genus *Verbascum* spread wild to many parts of Eurasia.

Verbascum genus (known as sığırkuyruğu in Turkish) belongs the Scrophulariaceae family are represented by 360 species around the world with remarkable diversity. 245 species are grown naturally in Türkiye with the 80% endemism ratio.^[6,7] *V. leiocarpum* is one of the local endemic plants growing in Erzincan, located in eastern Anatolia.

Many members of the genus were used in traditional medicine to treat a variety of diseases such as asthma, skin inflammation, hemorrhoids, arthritic wounds, fungal infections, and diarrhea.^[8] The anti-carcinogen,^[9] wound healing,^[10] anti-inflammatory,^[11] antihelmintic,^[12] anxiolytic, preanesthetic, sedative,^[13] antifungal and antioxidant,^[14] properties of *Verbascum* species have been reported in previous studies. The *Verbascum* species have many biologically active secondary metabolites such as saponins,^[15] flavonoids,^[16] iridoids,^[17] phenylethanoids,^[18] with varied biological activities. However, there were not found any reports about the chemical constituents and biological activity of *V. leiocarpum*.

To get further information about the phytochemical content of *V. leiocarpum*, the extract of the plant was subjected to chromatographic separation, which led to the isolation of a new compound named ferruginoside D (12) and fourteen additional known compounds. All isolated compounds were evaluated for antioxidant, α -glucosidase, and antiproliferative activities. The ferruginoside D (12) showed moderate antioxidant, good α -glucosidase inhibitory, and moderate antiproliferative activities.

Results and Discussion

Isolation and Characterization

Chromatographic separation of methanolic extract of *V. leiocarpum* led to isolation of catalpol (1),^[19] ajugol (2),^[20] eriodictyol (3),^[21] luteolin (4),^[22] and 10-O-cinnamoyl catalpol (5),^[23] and apigenin (6),^[24] 6-deoxy melittoside (7),^[25] poliumoside (8),^[26] verbascoside (9),^[27] isoverbascoside (10),^[28] ferruginoside C (11),^[29] ferruginoside D (12), harpagoside (13),^[30] *trans*-cinnamic acid,(14)^[31] and ursolic acid (15).^[32] The structures of the isolated molecules (Figure 1) were identified by comparing NMR data with those previous reports.

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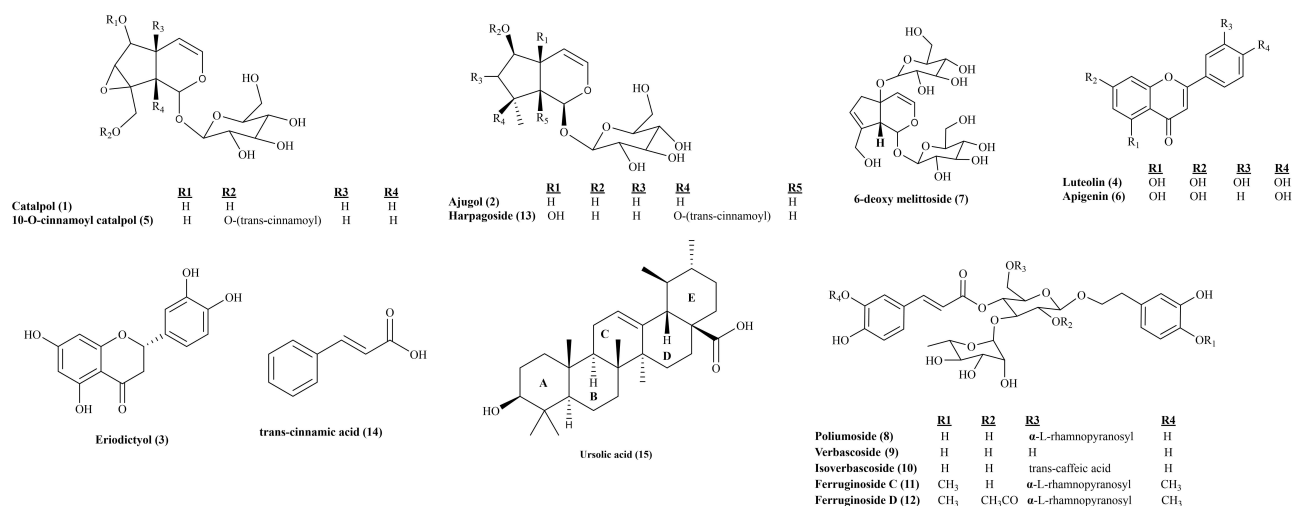


Figure 1. Chemical structures of the isolated compounds 1–15.

Ferruginoside D (12); The NMR data were given in Table 1. In the ¹H-NMR spectrum of the molecule, six aromatic proton signals were identified in the aromatic region revealing the presence of two trisubstituted benzene rings arranged in two ABX systems. Additionally, the AB system at δ_H 7.65 and 6.35 ppm as doublets (*J* = 15.9 Hz) indicated the presence of trans olefinic protons of the feruloyl group. Distinctive non-equivalent oxymethylene signals on the side chain of the aglycone were observed at δ_H 4.01 and 3.62 ppm, while a signal corresponding to benzyl methylene protons appeared at δ_H 2.71 ppm as multiplets. Furthermore, characteristic methoxy (–OCH₃) signals for feruloyl and 3-hydroxy-4-methoxyphenylethanol groups were evident at δ_H 3.86 (*s*, 3H) and 3.79 (*s*, 3H) ppm, respectively. Analysis of HETCOR, ¹³C, and HMBC spectra confirmed their attachment to carbons at δ_C 147.4 (C-3') and 145.3 (C-4), consistent with chemical shift values. Anomeric proton signals at δ_H 4.77 (*d*, *J* = 1.8 Hz, 1H, H-1'') and 4.60 (*d*, *J* = 1.8 Hz, 1H, H-1''') and methyl group signals at δ_H 1.18 (*d*, *J* = 6.2 Hz, 3H, H-6'') and 1.05 (*d*, *J* = 6.1 Hz, 3H, H-6''') indicated the presence of two 6-deoxy sugar units in the structure. A third sugar unit was confirmed by the anomeric proton signal at δ_H 4.52 (*d*, *J* = 8.2 Hz, 1H, H-1'). The three anomeric proton signals at δ_H 4.77 (*d*, *J* = 1.8 Hz, 1H, H-1''), 4.60 (*d*, *J* = 1.8 Hz, 1H, H-1'''), and 4.52 (*d*, *J* = 8.2 Hz, 1H, H-1') suggested the presence of β-glucose and two α-rhamnose units in the molecule.

Analysis of the ¹³C-NMR spectrum identified 39 carbon resonances, categorized as 6 quaternary (C), 23 methine (CH), 3 methylene (CH₂), 3 methyl (CH₃), 2 carbonyl (C=O), and 2 methoxy (OCH₃) carbons. The signal at δ_C 169.4 ppm indicated the presence of an acetyl group. The presence of another methyl signal at 1.96 (*s*, 3H) ppm confirmed this observation. Additionally, changes in the carbon resonance at δ_C 73.1 (C-2'') indicated the acetyl group's attachment to glucose's C-2'' carbon. The three anomeric carbon resonances at δ_C 101.4 (C-1'''), 100.3 (C-1'''), and 99.8 (C-1'') and the resonances at δ_C 65.3 (C-6'') for oxymethylene and δ_C 16.5 (C-6'') and 16.0 (C-6''') for

two methyl groups confirmed the presence of one glucose and two rhamnose units in the structure.

The connection patterns of aglycone and sugar units were constructed with the following HMBC interactions: H4'' (5.10) to C=O (165.8), H_{aa} (4.05) to C1'' (99.8), H3'' (4.04) to C1''' (101.4), H6'' (3.75) to C1'''' (100.3). The key HMBC correlations are given in Figure 2. The deep interpretation of NMR spectra of 12, was shown to close chemical shifts to ferruginoside C (11) except for one acetyl group.

The HMBC interaction of δ_H 1.96 with δ_C 73.1 showed the acetylation at the C2'' position. It can be supported by the down shielding of the C2'' signal of 12 about 1 ppm compared to the same position of 11. Thus compound 12 was elucidated as 2-acetyl-1-(3-hydroxy-4-methoxy-phenyl)-ethyl-O-(α-rhamnopyranosyl)-(1→3)-O-(α-rhamnosyl)-(1→6)-4-O-E-feruloyl-β-glucopyranoside. According to our best knowledge, the compound has not been reported in any plant species previously, and it was isolated and described from *V. leiocarpum* for the first time. It was named ferruginoside D because its chemical structure is identical to that of ferruginoside C. The corresponding spectra of 12 were given in Figure S25–S29.

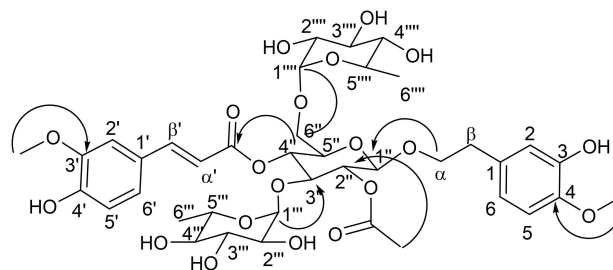


Figure 2. The key HMBC correlations of 12.

Table 1. ^1H -NMR (600 MHz) and ^{13}C -NMR (150 MHz) data of compound **12** in $\text{MeOH}-d_4$ (δ in ppm, J in Hz).

Position	12		
	^1H	^{13}C	HMBC
1	–	131.2	
2	6.66 (d, 2.1)	115.1	C1, C3, C β
3	–	148.9	
4	–	145.5	
5	6.80 (d, 8.2)	110.9	C1, C4, C6
6	6.62 (dd, 8.2; 2.0)	119.2	C1, C4, C5
α	4.05–3.91 (m) 3.62–3.55 (m)	69.8	C1'', C β
β	2.79–2.53 (m)	34.4	C1, C α
–OCH ₃	3.79 (s, 3H)	54.5	C4
1'	–	125.6	
2'	7.17 (d, 2.0)	109.8	C1', C3', C β'
3'	–	147.4	
4'	–	145.3	
5'	6.78 (d, 8.2)	114.5	C1', C4', C6'
6'	7.06 (dd, 8.2; 2.0)	122.4	C1', C4', C5'
α'	6.35 (d, 15.9)	113.0	C=O, C1', C β'
β'	7.65 (d, 15.9)	146.1	C1', C α'
C=O	–	165.8	
–OCH ₃	3.86 (s, 3H)	54.5	C3'
CH ₃ CO	–	169.4	
CH ₃ CO	1.96 (s, 3H)	18.9	C2''
1''	4.52 (d, 8.2)	99.8	C α , C2'', C3''
2''	5.10–4.84 (m)	73.1	C1'', C3''
3''	4.04–3.91 (m)	78.5	C1'''
4''	5.10–4.84 (m)	68.5	C=O
5''	3.68–3.62 (m)	72.8	C6''
6''	3.75–3.69 (m) 3.51–3.43 (m)	65.3	C1''''
1'''	4.77 (d, 1.8)	101.4	C3''
2'''	3.62–3.55 (m)	70.6	C1''', C3'''
3'''	3.84–3.81 (m)	70.0	
4'''	3.35–3.28 (m)	71.9	
5'''	3.51–3.43 (m)	69.9	
6'''	1.18 (d, 6.2)	16.0	
1''''	4.60 (d, 1.8)	100.3	C6''
2''''	3.68–3.62 (m)	70.3	
3''''	3.51–3.43 (m)	68.8	
4''''	3.26–3.21 (m)	71.6	
5''''	3.62–3.55 (m)	67.9	
6''''	1.05 (d, 6.1)	16.5	C5''''

Antioxidant Activity

In this study, the antioxidant potentials of the isolated compounds were determined using DPPH $^{\bullet}$ and ABTS $^{\bullet+}$ scavenging and the ferric reducing antioxidant power (FRAP) assays. The results were given in Table 2. Compounds **1**, **2**, **5**, **7**, **13**, **14**,

and **15** had a low antioxidant potential compared to other compounds and Trolox. The results showed that **9** and **10** had higher radical scavenging activity than Trolox and the other phenylethanoids (**8**, **11**, and **12**). It was concluded from the results the methoxylation of aromatic rings decreased the antioxidant activity. The antioxidant activities of **11** and **12** were

Table 2. Antioxidant activity results of isolated compounds (1–15).

Compounds	DPPH* (μM) IC ₅₀	ABTS* ⁺ (μM) IC ₅₀	FRAP (μg TE/g substance)
1	> 250	> 250	15.88 ± 0.21 ^[a,b]
2	> 250	> 250	13.44 ± 0.24 ^[a,b]
3	68.09 ± 3.68 ^[b]	30.05 ± 1.95 ^[a,b,c]	88.32 ± 0.56 ^[e]
4	24.03 ± 1.72 ^[a]	14.53 ± 1.60 ^[a,b]	153.52 ± 0.49 ^[f]
5	181.05 ± 1.11 ^[c]	161.38 ± 1.81 ^[d]	27.83 ± 0.12 ^[b,c]
6	69.05 ± 0.57 ^[b]	33.70 ± 2.84 ^[a,b,c]	71.41 ± 0.75 ^[d,e]
7	> 250	> 250	2.46 ± 0.32 ^[a]
8	58.49 ± 0.20 ^[b]	20.93 ± 1.30 ^[a,b]	111.08 ± 0.74 ^[e]
9	17.08 ± 1.66 ^[a]	10.72 ± 2.80 ^[a]	258.32 ± 0.37 ^[h]
10	20.83 ± 1.67 ^[a]	12.90 ± 2.28 ^[a,b]	179.05 ± 0.68 ^[g]
11	85.28 ± 2.21 ^[b]	42.43 ± 2.12 ^[b,c]	57.83 ± 1.00 ^[d]
12	78.99 ± 1.31 ^[b]	53.45 ± 2.36 ^[c]	38.15 ± 0.71 ^[c]
13	> 250	> 250	26.28 ± 0.55 ^[b,c]
14	> 250	> 250	9.46 ± 0.67 ^[a,b]
15	> 250	> 250	3.93 ± 0.61 ^[a]
Trolox	25.01 ± 1.12 ^[a]	26.72 ± 0.84 ^[b]	–

*IC₅₀ values are expressed as mean ± standard deviation of three independent measures. **[a–h]: represents a statistically significant difference in the same column ($p < 0.05$).

first reported in this study. The compounds were found moderately active in all antioxidant activity assays. The flavonoid aglycons, (3, 4, and 6) were found to be potent radical scavenging and FRAP activity. The results indicate that 4, 9, and 10 can be potential molecules for treating oxidative stress-related illnesses.

Antiproliferative Activity

A significant part of the chemotherapeutic agents with different chemical structures, which have been developed to be used in cancer treatment still do not show a therapeutic feature at the desired level. In addition, due to the resistance mechanisms developed against chemotherapeutic agents for various reasons and their undesirable side effects, their already insufficient therapeutic power is decreasing day by day. At this point, evaluating the possible anticancer or adjuvant effects of naturally derived metabolites that can be isolated from different sources is very important in terms of increasing the number of promising molecules that can be used in cancer therapy. All isolated compounds (1–15) were evaluated for their cytotoxicity against multiple cell lines using the MTT test (Table 3). Accordingly, when the antiproliferative effects of these compounds for glioma cell lines (A172 and C6) were examined IC₅₀ values, it was determined that all compounds (IC₅₀ value, 2.5–278.6 μM), except for 14, exhibited a powerful anti-cancer effect. The compounds, except for 3 and 14, exhibited a strong antiproliferative effect (IC₅₀ value, 53.14–423.59 μM) against gynecologic cancer cells (HeLa and A2780). The IC₅₀ values of all compounds, except for 2 and 14, were close to the control

anticancer drug, 5FU, in the colon (SW620 and HT29) cancer cells (IC₅₀ value, 49.16–410.72 μM). When the IC₅₀ values of these compounds for normal cell lines were investigated, it was stressed that compounds 1, 3, 4, 6, and 13 (IC₅₀ value, 223.08–430.29 μM) for Beas2B, compound 4 (IC₅₀ value, 434.04 μM) for RPE, and compounds 1 and 4 (IC₅₀ value, 245.63–320.29 μM) for HSF were safe considering the control anticancer drug, 5FU (IC₅₀ value, 231.25, 533.37, and 234.71 μM, respectively).

The antiproliferative activities of 11 and 12 were first reported in this study. Compound 12 had highly cytotoxic activity against the normal lung cell line, Beas2B, while moderate activity against the other cell lines tested. Low IC₅₀ values indicate that the test substances have more antiproliferative, which is also desirable. In general, when IC₅₀ values were examined, antiproliferative effects of 1, 3, 4, 6, and 13 against cancer cell lines indicate that they are suitable candidates for further pharmacological tests (Table 3). So these values are much better in the control drug, 5FU. This finding indicates that the tested compounds were cancer-specific. In this case, it may be recommended to direct the compounds to further studies as they contain molecules that can be used in cancer research.

The Effect of The Compounds on The Morphology of The Cells

In this study, the effects of compounds 1, 3, and 8 on A172, A2780, HeLa, and Beas2B cell morphology were observed and photographed. As shown in Figure 3, significant morphological changes were recorded in the treated cells with compounds 1, 3, and 8 in comparison to the untreated cells. Some of these

Table 3. Antiproliferative activity of compounds 1–15 against cell lines.

Compounds	Beas2B**	RPE**	HSF**	A172**	IC ₅₀ (μM)		HeLa**	A2780**	SW620**	HT29**
					C6**					
1	279.74 ± 3.1 ^[a]	256.20 ± 2.6 ^[d]	245.63 ± 2.7 ^[g]	180.58 ± 1.7 ^[c,d,e]	143.72 ± 1.8 ^[e,f,g]	152.85 ± 2.9 ^[c,d,e]	173.73 ± 1.7 ^[c]	329.78 ± 2.7 ^[e]	273.17 ± 2.8 ^[d]	
2	290.8 ± 0.5 ^[a]	287.21 ± 2.6 ^[d,e]	191.33 ± 2.7 ^[f]	196.79 ± 1.7 ^[d,e,f]	168.25 ± 1.9 ^[f,g]	166.70 ± 2.9 ^[e]	190.90 ± 1.7 ^[c]	636.49 ± 3.8 ^[g]	461.75 ± 3.4 ^[f]	
3	296.55 ± 2.4 ^[e]	329.05 ± 2.8 ^[e]	198.86 ± 1.7 ^[f]	278.61 ± 1.8 ^[f]	223.56 ± 2.4 ^[h]	442.91 ± 3.0 ^[h]	531.73 ± 2.8 ^[e]	129.89 ± 1.5 ^[a,b,c,d]	49.16 ± 0.6 ^[a]	
4	350.34 ± 3.1 ^[f]	434.04 ± 3.4 ^[f,g]	320.29 ± 2.4 ^[j]	224.88 ± 1.9 ^[e,f]	191.24 ± 1.9 ^[a,b]	113.54 ± 1.5 ^[b,c,d]	155.50 ± 1.9 ^[b,c]	153.26 ± 1.6 ^[a,b,c,d]	115.95 ± 1.1 ^[b]	
5	15.47 ^[a]	309.11 ± 2.9 ^[d,e]	153.14 ± 2.6 ^[e]	189.81 ± 1.9 ^[c,d,e]	165.59 ± 2.1 ^[f,g]	167.99 ± 3.0 ^[e]	102.28 ± 2.2 ^[a,b]	188.07 ± 2.9 ^[c,d]	194.65 ± 2.9 ^[c]	
6	430.29 ± 3.3 ^[g]	387.48 ± 2.9 ^[f]	167.38 ± 1.5 ^[e,f]	140.79 ± 1.1 ^[b,c,d,e]	71.10 ± 1.0 ^[b,c,d]	396.26 ± 2.9 ^[g]	423.59 ± 2.0 ^[d]	241.40 ± 1.7 ^[d]	103.20 ± 1.0 ^[a,b]	
7	75.74 ± 1.1 ^[b]	261.25 ± 3.1 ^[d]	157.71 ± 2.0 ^[e]	159.14 ± 2.0 ^[b,c,d,e]	120.87 ± 2.1 ^[d,e,f]	108.78 ± 2.3 ^[b,c]	148.07 ± 1.9 ^[b,c]	410.72 ± 3.3 ^[f]	296.77 ± 3.5 ^[d]	
8	5.66 ^[a]	128.36 ± 2.9 ^[b]	174.60 ± 3.0 ^[e,f]	103.75 ± 1.8 ^[a,b,c,d]	78.65 ± 2.0 ^[c,d]	87.80 ± 2.8 ^[a,b]	101.70 ± 1.8 ^[a,b]	183.67 ± 3.4 ^[b,c,d]	126.97 ± 2.5 ^[b]	
9	19.16 ± 0.6 ^[a]	106.60 ± 2.8 ^[a,b]	8.49 ^[a]	43.58 ± 1.8 ^[a]	106.31 ± 2.0 ^[d,e]	94.67 ± 2.7 ^[a,b]	70.25 ± 1.8 ^[a]	118.49 ± 3.0 ^[a,b]	121.63 ± 2.9 ^[b]	
10	97.22 ± 2.8 ^[b]	459.29 ± 4.8 ^[g]	18.25 ± 2.7 ^[a,b]	27.57 ± 0.9 ^[a]	2.50 ^[a]	68.00 ± 1.6 ^[a,b]	71.95 ± 1.8 ^[a]	121.46 ± 2.5 ^[a,b,c]	114.75 ± 2.0 ^[b]	
11	6.45 ± 0.8 ^[a]	107.19 ± 1.9 ^[a,b]	88.16 ± 2.1 ^[d]	80.66 ± 1.3 ^[a,b]	88.08 ± 3.8 ^[c,d,e]	105.37 ± 3.1 ^[a,b,c]	67.89 ± 2.8 ^[a]	106.44 ± 2.9 ^[a]	115.71 ± 2.8 ^[b]	
12	3.81 ± 0.7 ^[a]	93.30 ± 1.0 ^[a,b]	66.34 ± 0.5 ^[c,d]	71.80 ± 2.1 ^[a,b]	74.96 ± 0.9 ^[c,d]	86.14 ± 1.1 ^[a,b]	53.14 ± 1.2 ^[a]	99.94 ± 2.0 ^[a]	101.47 ± 1.4 ^[a,b]	
13	223.08 ± 3.5 ^[d]	185.32 ± 3.0 ^[c]	41.96 ± 1.1 ^[b,c]	84.37 ± 1.8 ^[a,b]	17.01 ^[a,b]	158.93 ± 2.1 ^[d,e]	174.66 ± 2.7 ^[c]	162.43 ± 2.2 ^[a,b,c,d]	132.04 ± 2.0 ^[b]	
14	537.46 ± 2.9 ^[h]	388.57 ± 1.9 ^[f]	277.67 ± 2.5 ^[h]	1830.32 ± 5.3 ^[h]	1105.70 ± 3.8 ^[j]	732.18 ± 2.8 ^[i]	917.05 ± 3.1 ^[f]	1037.19 ± 2.9 ^[h]	909.15 ± 2.8 ^[g]	
15	163.74 ± 2.8 ^[c]	55.59 ± 1.0 ^[a]	8.28 ^[a]	118.81 ± 2.1 ^[a,b,c,d]	36.70 ± 0.9 ^[a,b,c,d]	55.77 ± 1.2 ^[a]	61.51 ± 1.1 ^[a]	108.84 ± 2.0 ^[a]	99.67 ± 1.4 ^[a,b]	
5FU	231.25 ± 1.3 ^[d]	533.37 ± 2.7 ^[h]	234.71 ± 2.4 ^[g]	360.86 ± 2.6 ^[g]	342.95 ± 2.3 ^[i]	293.75 ± 1.6 ^[f]	440.04 ± 1.4 ^[d]	322.81 ± 1.7 ^[e]	397.22 ± 1.3 ^[e]	

*IC₅₀ values are expressed as mean ± standard deviation of three independent measures. **[a–j]: represents a statistically significant difference in the same column (*p* < 0.05). ***If IC₅₀ values is smaller than 10, the SD value is < 0.5.

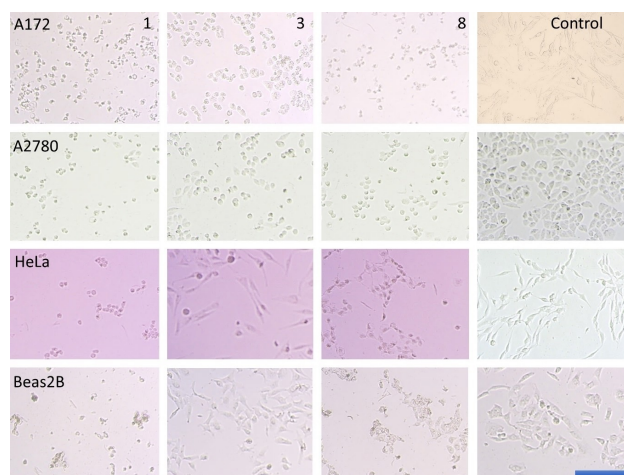


Figure 3. Effects of test isolates on cancer cell line morphology.

alterations like shrinkage, cytoplasmic blebs, and anomalous globular structure; are all hallmarks of apoptotic cell death. The results in Figure 3 have also shown that most of the treated cells had an abnormal and detaching fibroblast-like appearance.

The above-mentioned changes in the cell morphology of the cells occur due to the given low-concentration compounds indicating that they exhibit their apoptotic activities by changing the shape of the cell.

Cytotoxicity Test

The effects of the new molecules on membrane integrity can be implied by some information about their cytotoxic activities. For this, the activity of cytoplasmic lactate dehydrogenase (LDH) leaking from the damaged plasma membrane to the environment is measured with the help of a kit. LDH activity indicates the amount of leakage implied indirect membrane damage. In this study, cytotoxicity induced by the molecules was measured using IC_{50} concentrations. Accordingly, the compounds that were found to be effective in the MTT proliferation test showed different cytotoxicity values varying between about 13–16% against normal cell lines at IC_{50} concentration, while the less effective ones showed cytotoxicity in the range of only 9–12%. When the cytotoxic effects of the tested molecules on cancer cells were examined, the molecules that were found to be effective in the MTT proliferation test showed different cytotoxicity values, varying in the range of about 14–16% against cancer cell lines at IC_{50} concentration, while the less effective ones only revealed cytotoxicity in the range of 9–13% (Table 4). If the MTT results, which show the antiproliferative power, and the LDH findings, which reveal the cytotoxic feature, were evaluated, it was strongly stated that these new molecules show the most optimal antiproliferative and cytotoxic effect against cancer and normal cells compared to the positive control, 5FU. This means that at the IC_{50} concentration, these molecules are sufficiently toxic to cancer

cell lines but safe against normal cells, providing an opportunity for further preclinical studies.

α -glucosidase Inhibition Activity

The α -glucosidase enzyme inhibition properties of isolated compounds are given in Table 5. Among the isolated compounds 1, 2, 5, 7, 13, and 14 showed the lowest activity compared to acarbose (22.82 μ M). The α -glucosidase inhibitory effects of isolated phenylethanoids, (8, 9, 10, 11, and 12) were found in a range of 3.12 ± 0.80 – 19.93 ± 1.30 μ M. Compounds 9 and 10, bearing hydroxyl groups on the aromatic rings, exhibited prominent activity, whereas compounds 11, and 12, bearing methoxy groups, displayed relatively lower activity. According to the results, the presence of hydroxyl groups on the aromatic rings of phenylethanoids was the key function for the inhibition of α -glucosidase. Similar trends in the structure-activity relationship of phenylethanoids were reported previously.^[33,34] Upon comparing the inhibitory activities of molecules 8, 9, and 10, possessing identical aromatic cores, the incorporation of an additional rhamnose group in 8 resulted in a notable decrease in activity. Therefore, it can be inferred that the bioactivity of glycosylated compounds varies based on the number of sugar moieties attached to the structure. The remarkable difference in the activities of compounds 9 and 10 implies that the positioning of the sugar groups on different sites is also a factor in the modulation of activity. Furthermore, this study reports for the first time on the α -glucosidase inhibitory activity of ferruginoside C (11) and its 2''-acetylated form, ferruginoside D (12). The presence of the acetyl group slightly contributed to the α -glucosidase inhibitory effect.

These findings shed light on the structure-activity relationship of phenylethanoids and provide insight into the potential use of these compounds for the treatment of type 2 diabetes. The flavonoids, 3 and 6 have exhibited a moderate level of inhibition activity, with IC_{50} values of 32.05 ± 1.95 μ M and 28.20 ± 1.75 μ M, respectively. On the other hand, 4 has shown good inhibition activity against α -glucosidase with an IC_{50} value of 13.93 ± 0.40 μ M. Many studies have reported that flavonoids inhibit the α -glucosidase supporting our results.^[35–39] Therefore, these compounds may have potential therapeutic effects in managing diabetes and other conditions associated with high blood sugar levels, as α -glucosidase inhibitors can slow down the digestion of carbohydrates and prevent a rapid increase in blood glucose levels after meals.

Conclusions

The phenylethanoids and flavonoids were found to have antioxidant and α -glucosidase inhibitory activity. It was demonstrated clearly from the anticancer results that some iridoids (1, 2, 5, 7, and 13), flavonoids (4 and 6), and phenylethanoids (8 and 10) exhibited more antiproliferative effects against cancer cell lines than normal cell lines (Beas2B, RPE, and HSF). When the sensible outcomes of the assays were considered together,

Table 4. % Cytotoxicity of 1–15 at IC₅₀ concentrations against the cells.

Compounds	Beas2B**	RPE**	HSF**	A172**	C6**	HeLa**	A2780**	SW620**	HT29**
1	10.3 ± 0.8 ^[a,b,c,d]	10.0 ± 0.7 ^[a,b]	11.3 ± 0.9 ^[a,b,c]	15.6 ± 1.4 ^[a]	15.1 ± 1.3 ^[d,e,f,g]	12.9 ± 1.0 ^[c,d,e,f]	13.2 ± 1.2 ^[b,c,d]	10.6 ± 0.8 ^[a]	12.1 ± 1.0 ^[a,b,c]
2	14.7 ± 1.2 ^[f]	10.7 ± 0.7 ^[a,b]	11.7 ± 0.9 ^[a,b,c]	15.4 ± 1.3 ^[d]	15.7 ± 1.4 ^[e,f,g]	13.1 ± 1.1 ^[d,e,f,g]	15.7 ± 1.2 ^[d,e]	13.2 ± 1.3 ^[b,c,d,e]	11.3 ± 0.9 ^[a,b,c]
3	11.7 ± 0.9 ^[b,c,d,e]	10.4 ± 0.7 ^[a,b]	11.4 ± 0.8 ^[a,b,c]	11.0 ± 0.9 ^[a,c,d]	10.6 ± 0.8 ^[a]	10.6 ± 0.6 ^[a,b,c]	9.9 ± 0.8 ^[a]	15.9 ± 1.3 ^[a]	15.8 ± 1.3 ^[f]
4	12.1 ± 1.0 ^[c,d,e]	11.6 ± 0.7 ^[b]	11.6 ± 0.9 ^[a,b,c]	16.0 ± 1.3 ^[g]	16.3 ± 1.3 ^[g]	15.3 ± 1.3 ^[g,h]	16.7 ± 1.4 ^[e]	16.0 ± 1.4 ^[g]	14.9 ± 1.3 ^[e,f]
5	13.3 ± 1.1 ^[e,f]	11.3 ± 0.8 ^[a,b]	10.2 ± 0.7 ^[a,b,c]	11.7 ± 0.9 ^[a,b,c,d]	13.2 ± 1.1 ^[a,b,c,d]	9.7 ± 0.7 ^[a]	11.4 ± 0.8 ^[a,b]	11.9 ± 0.8 ^[a,b,c]	12.7 ± 1.1 ^[b,c,d,e]
6	10.8 ± 0.8 ^[a,b,c,d]	11.1 ± 0.8 ^[a,b]	9.9 ± 0.7 ^[a,b,c]	14.1 ± 1.2 ^[d,e,f,g]	15.8 ± 1.2 ^[e,f,g]	10.4 ± 0.8 ^[a,b]	11.6 ± 0.9 ^[a,b]	16.3 ± 1.3 ^[g]	15.1 ± 1.4 ^[e,f]
7	10.2 ± 0.7 ^[a,b,c,d]	11.2 ± 0.8 ^[a,b]	10.2 ± 0.8 ^[a,b,c]	10.6 ± 0.8 ^[a,b]	16.0 ± 1.3 ^[e,f,g]	14.0 ± 1.2 ^[e,f,g,h]	11.7 ± 0.9 ^[a,b]	10.9 ± 0.6 ^[a,b]	12.9 ± 1.0 ^[b,c,d,e]
8	14.8 ± 1.2 ^[f]	9.9 ± 0.6 ^[a,b]	9.6 ± 0.7 ^[a]	12.1 ± 1.1 ^[b,c,d]	15.9 ± 1.2 ^[e,f,g]	15.7 ± 1.3 ^[h]	15.4 ± 1.4 ^[d,e]	12.0 ± 1.0 ^[b,c]	10.7 ± 1.3 ^[a,b]
9	15.1 ± 1.3 ^[f]	14.0 ± 1.2 ^[d]	12.0 ± 1.0 ^[b,c,d]	9.5 ± 0.6 ^[a]	11.5 ± 0.9 ^[a,b,c]	14.8 ± 1.2 ^[f,g,h]	16.2 ± 1.3 ^[e]	13.5 ± 1.2 ^[c,d,e,f]	10.1 ± 0.8 ^[a]
10	9.9 ± 0.6 ^[a,b,c]	9.3 ± 0.8 ^[a]	14.3 ± 1.2 ^[e]	15.2 ± 1.2 ^[f,g]	16.1 ± 1.4 ^[f,g]	14.9 ± 1.2 ^[f,g,h]	14.9 ± 1.3 ^[c,d,e]	13.9 ± 1.1 ^[c,d,e,f]	13.4 ± 1.1 ^[c,d,e,f]
11	12.4 ± 1.3 ^[d,e]	11.2 ± 0.6 ^[a,b]	10.2 ± 0.9 ^[a,b,c]	11.7 ± 0.7 ^[a,b,c,d]	13.4 ± 1.2 ^[b,c,d,e]	11.8 ± 1.2 ^[a,b,c,d]	11.4 ± 1.3 ^[a,b]	11.7 ± 0.8 ^[a,b,c]	12.3 ± 1.2 ^[a,b,c,d]
12	11.3 ± 0.7 ^[a,b,c,d]	10.4 ± 0.8 ^[a,b]	9.7 ± 0.6 ^[a,b]	11.5 ± 0.5 ^[a,b,c]	12.8 ± 1.3 ^[a,b,c,d]	11.7 ± 1.4 ^[a,b,c,d]	11.2 ± 1.2 ^[a,b]	10.8 ± 0.6 ^[a,b]	12.1 ± 1.1 ^[a,b,c]
13	9.4 ± 0.7 ^[a]	9.7 ± 0.6 ^[a,b]	13.9 ± 1.2 ^[d,e]	14.9 ± 1.2 ^[e,f,g]	15.9 ± 1.3 ^[e,f,g]	11.1 ± 0.9 ^[a,b,c,d]	12.8 ± 1.1 ^[b,c]	11.4 ± 0.8 ^[a,b,c]	13.7 ± 1.2 ^[c,d,e,f]
14	9.5 ± 0.5 ^[a,b]	13.6 ± 1.1 ^[c,d]	14.2 ± 1.2 ^[e]	12.7 ± 1.1 ^[b,c,d,e]	10.8 ± 0.8 ^[a,b]	11.3 ± 0.9 ^[a,b,c,d]	10.8 ± 0.8 ^[a,b]	12.3 ± 1.0 ^[a,b,c,d]	12.9 ± 1.0 ^[b,c,d,e]
15	11.8 ± 0.8 ^[c,d,e]	14.8 ± 1.2 ^[d]	15.2 ± 1.3 ^[e]	14.7 ± 1.2 ^[e,f,g]	15.8 ± 1.3 ^[e,f,g]	14.6 ± 1.2 ^[f,g,h]	14.7 ± 1.2 ^[c,d,e]	15.2 ± 1.3 ^[e,f,g]	14.7 ± 1.3 ^[d,e,f]
5FU	12.4 ± 1.1 ^[d,e]	11.9 ± 0.9 ^[b,c]	12.1 ± 1.0 ^[c,d]	13.4 ± 1.1 ^[c,d,e,f]	14.1 ± 1.2 ^[c,d,e,f]	12.8 ± 1.1 ^[b,c,d,e]	14.3 ± 1.2 ^[c,d,e]	14.6 ± 1.2 ^[d,e,f,g]	13.8 ± 1.1 ^[c,d,e,f]

*Percent cytotoxicity was expressed as mean ± standard deviation of three independent measures. **[a–h]: represents a statistically significant difference in the same column (*p* < 0.05).

Table 5. α -glucosidase inhibition activity of the isolated compounds (1–15).

Compounds	IC ₅₀ (μ M)
1	> 250
2	> 250
3	32.05 $\pm$ 1.95 ^[c]
4	13.93 $\pm$ 0.40 ^[b]
5	> 250
6	28.20 $\pm$ 1.75 ^[c]
7	> 250
8	15.93 $\pm$ 1.30 ^[b]
9	3.12 $\pm$ 0.80 ^[a]
10	5.60 $\pm$ 0.28 ^[a]
11	19.93 $\pm$ 1.30 ^[b]
12	17.43 $\pm$ 2.12 ^[b]
13	> 250
14	> 250
15	6.60 $\pm$ 0.50 ^[a]
Acarbose	22.82 $\pm$ 0.84 ^[c]

*IC₅₀ values are expressed as mean $\pm$ standard deviation of three independent measures. **[a–c]: represents a statistically significant difference ($p < 0.05$).

we strongly recommend their use in further clinical studies since the isolates have sufficient anticancer properties with the help of their apoptotic potency against cancer cells and low cytotoxicity against normal cells. Consequently, our preliminary results depicted that these compounds are potentially significant suitable anticancer candidates and deserve further preclinical and clinical processes.

Experimental Section

General Experimental Procedure

Bruker Avance II (400 MHz) and Agilent DD2 (600 MHz) spectrometers were used to record the NMR spectra, and an Agilent 6210 LC-TOF/MS fitted with a ZORBAX SB–C18 column (4.6 $\times$ 100 mm, 2.7 μ m) was used to record the mass spectra. An Elisa microplate reader (Epoch-2) was used to absorbance readings. Sephadex LH-20 was used for open-column chromatography (CC). A Buchi Reveleris X2 flash chromatography (FC) instrument equipped with a Buchi Ecoflex C18 column (23 $\times$ 400 mm, 43–60 μ m) was used for flash chromatographic separations. The specific optical rotation of compound 12 was measured using a WZZ-3 polarimeter. Thermo Scientific FT-IR Nicolet 6700 spectrophotometer was used for the recording of IR spectra. Solvents were purchased from Sigma-Aldrich. TLC analysis was achieved on precoated silica gel 60 GF254 plates, and the spots were visualized by spraying with 10% sulfuric acid in ethanol and/or UV 254 and 366.

Plant Material

V. leiocarpum was collected from Iliç-Erzincan- Türkiye. The plant samples were authenticated by Prof. Dr. Ali KANDEMİR and the

voucher specimen was deposited in the Erzincan Binali Yıldırım University Herbarium (EBYU) under Kandemir 10519 number on 20 May 2020.

Extraction and Isolation

The aerial parts of *V. leiocarpum* (1 kg) were extracted with MeOH: water (70:30) twice overnight. Filtered solvents were evaporated reducing pressure to give a dark brown slurry crude extract (20 g). The extract was fractionated via Flash chromatography (FC) using MeOH (A): water (B) mixture with increasing methanol from 0:10 (A: B) to 10:0 (A: B) on the 330 g pre-packed C18 column. 106 fractions were collected. According to the TLC basis, seven subfractions were obtained: fracs 4–7 (VG1), 8–12 (VG2), 13–31 (VG3), 32–40 (VG4), 41–77 (VG5), 78–87 (VG6), and 88–106 (VG7). VG3 was applied to Sephadex LH-20 column chromatography (CC) using MeOH as the mobile phase. 49 fractions were collected and combined as follows: Fracs 1–19 (VG3-1), 20–30 (VG3-2), 31–42 (VG3-3), and 43–49 (VG3-4). VG3-2 was further purified over silica gel CC using EtOAc: MeOH (9:1) to obtain 1 (32 mg). VG4 chromatographed over Sephadex LH-20 using a MeOH: water (1:1) system. The fractions were combined to give 3 subfractions (Frcs 1–11, 12–21, and 29–37). The 12–21th fractions gave 2 (78 mg). 29–37 frcs were separated CC over silica gel using EtOAc: MeOH: CHCl₃ (4:2:2) to afford 3 (15 mg). VG5 separated using Sephadex LH-20 using MeOH. Fracs 1–17, 18–33, 36–49, and 50–54 were combined according to TLC analyses. The fractions 50–54 were applied to silica gel CC to give 4 (12 mg) using EtOAc: MeOH: CHCl₃ (4:2:2). VG6 was purified in latter conditions to afford 5 (30 mg).

As mentioned above, the above-ground parts of the plant were extracted to obtain 24 g crude extract. The extract was first fractionated using FC over a C18-packed column with increasing MeOH. The collected 126 fracs were combined according to TLC analysis and detection pattern at 330 and 280 in 11 subfractions (VK1 to VK11). VK6 was applied to Sephadex LH-20 using MeOH as the mobile phase to afford three subfractions. Separation of subfraction 3 using silica gel CC with CHCl₃:MeOH (8:2) system gave 6 (50 mg). VK7 was further purified with Sephadex LH-20 CC in a MeOH: water mixture. The purification of 7 (15 mg) was achieved. VK8 was fractionated to give two fractions with Sephadex LH-20 in methanol. Frac 1 and 2 were individually purified using preparative-HPLC (p-HPLC) in the following conditions: mobile phase: 65:35 (water: MeOH) at 8 ml/min at 235 and 280 nm, column: C18 (20 $\times$ 250 mm, 10 μ m). The p-HPLC application of Fr-1 was given at RT = 23 min 8 (10 mg) and of Fr-2 was given at RT = 18 min 9 (100 mg). VK9 was applied to p-HPLC using above mentioned chromatographic conditions to give 10 (35 mg) at RT = 32–34 min. The collected peaks RT = 8–12 minutes were combined and further purified over silica CC using EtOAc:CHCl₃ system to afford 11 (11 mg). VK10 fractionated over Sephadex LH-20 using MeOH as mobile phase. Four subfractions (Frcs 10–20, 21–28, 29–35, and 36–45) were obtained. Fracs 21–28 was applied to Sephadex LH-20 under the above conditions. The fractions were combined into two groups (fracs 12–16 and 17–21). Group 1 chromatographed using silica gel CC in EtOAc:CHCl₃ mixture. The elution achieved purification of 12 (6 mg). Group 2 was purified on silica gel CC with CHCl₃ to afford 13 (9 mg). The fractions of 29–35 of VK10 were applied to silica gel CC using hexane: EtOAc (8:2). The separation of 14 (15 mg) was achieved from this process. VK-11 was separated using normal phase-p-HPLC using hexane: EtOAc (6:4). The elution was monitored at 225 and 235 nm. The collected peak at RT 12–14 min gave 15 (45 mg). Descriptive isolation flowcharts were given in Figure S1 and Figure S2.

DPPH Assay

The isolated compounds' free radical (DPPH[•]) scavenging abilities were tested using a modified version of the procedure described by.^[40] DPPH[•] (2,2-diphenyl-1-picryl hydrazine) solution was prepared at 0.26 mM in methanol. Stock solutions (1 mg/ml) of compounds and standards were prepared. Samples at 20, 40, 80, 120, 160, 240, 320, and 400 μ l amounts were added to test tubes, and the final volume was completed to 3 ml with methanol. Then 0.26 mM DPPH[•] solution (1.0 ml) was added to each. The resulting mixture was vortexed and incubated at room temperature for 30 minutes. At the end of the time, the absorbance of each reaction mixture was measured at 517 nm. The absorbance values were converted to % activity, the IC₅₀ (μ M) for each compound was calculated, and the results were compared with the standard antioxidant Trolox.

ABTS Assay

Cationic radical scavenging activities of compounds isolated from plant material were determined by some minor modifications of the method reported by.^[41] 2 mM ABTS (2,2'-Azino-bis 3-ethyl-benzothiazoline-6-sulfonic acid) solution and 2.45 mM K₂S₂O₈ (potassium persulfate) solution were prepared in 0.1 M pH:7.4 phosphate buffer. Then these two solutions were mixed at a ratio of 1:2 and kept in the dark for 12 hours. The compounds and standards to be tested were prepared as stock solutions (1 mg/ml). Samples were taken in amounts of 20–400 μ l, and their volumes were made up to 3 ml with phosphate buffer. 1 ml of ABTS^{•+} solution was added. The mixture was mixed by vortexing and incubated for 60 minutes at room temperature. The absorbance of each reaction mixture at 734 nm was measured and the obtained absorbance values were converted to % activity. Each sample's IC₅₀ (μ M) was calculated, and the results were compared with Trolox as the reference substance.

FRAP Activity

The ferric-reducing antioxidant power activity test was performed with some modifications to the method reported by.^[42] For testing, stock solutions of the samples were prepared, and 100 μ l of these solutions were placed in test tubes. The volume was made up to 1.25 ml with phosphate buffer (0.2 M, pH 6.6). Following the addition of 1.25 ml of potassium ferric cyanide [K₃Fe(CN)₆] (1%) to this mixture, the mixture was incubated for 20 minutes at 50 °C. After incubation, 1.25 ml of 10% trichloroacetic acid (TCA) and 0.25 ml of 0.1% FeCl₃ solution were added to the reaction medium, and the absorbance of the final mixture was measured at 700 nm. The result was expressed as μ g Trolox equivalent in g substance (μ g TE/g substance).

α -glucosidase Inhibition Assay

The α -glucosidase inhibitory effect of isolated molecules was determined using the method of.^[43,44] Firstly, the α -glucosidase enzyme (0.1 Unit/ml) was dissolved in phosphate buffer (0.1 M, pH 6.8) and this solution was used as an enzyme solution in the study. Then, *p*-nitrophenyl- α -D-glucopyranoside (pNPG 0.375 mM) was prepared in the same buffer. 10 μ l of the enzyme solution and 20 μ l of the test sample were mixed. 20 μ l of the substrate was added to the mixture and incubated for 30 minutes at 37 °C. Following incubation, 50 μ l (0.2 M) sodium carbonate was added to the medium, and the absorbances of the final mixture were measured at 405 nm. Each sample's IC₅₀ (in μ M) value was calculated and the results were compared with the positive control acarbose.

Antiproliferative Assay

In this study, A172 (ATCC, CRL-1620) and C6 (ATCC, CCL-107) brain cancer cell lines, HeLa (ATCC, CCL-2) and A2780 (RRID, CVCL-0134) cervix and ovarian cancer cell lines, SW620 (ATCC, CCL-227) and HT29 (ATCC, HTB-38) colon cancer cell lines, and Beas2B (ATCC, CRL-9609), RPE (ATCC, CRL-4000), and HSF (ATCC, CRL-7449) normal cell lines, respectively lung, retinal, and skin, were used. All cell preparation procedures were carried out in a sterile environment in a laminar cabinet. The cell lines were used after they were confluent in 37 °C, 5% CO₂ conditions in the supplemented DMEM or RPMI1640 medium containing 10% FBS (Fetal Bovine Serum) and 2% PenStrep (Penicillin-Streptomycin) solution. Measuring plates were seeded with 10,000 cells per well. After approximately 16 hours of pre-incubation, test molecules were added and measurements were performed after 24 hours of incubation. MTT [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide] test was used to measure the effects of the isolated test molecules on cell proliferation. The results were reported as % cell inhibition and the optical density of the solvent (DMSO) treated cells was assumed to be 100%. Accordingly, the %inhibition was calculated according to the formula $[1 - (A \text{ test substance} / A \text{ solvent control})] \times 100$. The MTT method was used on cells with increasing concentrations of each test substance (1.96, 3.91, 7.81, 15.63, 31.25, 62.5, and 125.0 μ g/ml) over a specified range to determine the IC₅₀ concentrations of test substances.

Cytotoxicity Test

Whether the test substances to be tested were cell cytotoxic or cytostatic was determined by the LDH method. An increase in the number of cells that die during the incubation period, depending on the substance tested, will result in an increase in LDH in the culture supernatant. Lactate dehydrogenase (LDH) is a cytoplasmic enzyme that is found in most cells and is stable. For this purpose, the LDH cell cytotoxicity kit was used according to the manufacturer's procedure. Briefly, the change in the amount of formazan formed as a result of LDH enzyme activity was measured and evaluated according to the formula below; %Cytotoxicity = $[(\text{Substance Absorbance} - \text{Low Control}) / (\text{High Control} - \text{Low Control})] \times 100$.

Statistical Analysis

Statistical differences between groups were determined using analysis of variance (ANOVA) and multiple comparisons tests (post hoc Duncan's test) ($p \leq 0.05$). The results were reported as mean values $\pm$ SDs of three independent assays.

Ferruginoside D (12)

Yellow amorphous solid; $[\alpha]_{24}^D -13.2$ (c 2.00, MeOH); UV (MeOH) λ_{max} 336, 284 nm, (log ϵ): 358 (2.00), IR (KBr) ν_{max} 3412, 2919, 2850, 1633, 1608, 1508, 1467, 1384, 1324, 1290, 1237, 1205, 1179, 1127, 1071, 989, 927, 816, 703 and 554 cm⁻¹. The molecular formula was established C₃₉H₅₂O₂₀ based on LC-TOF/MS signal at m/z [M-H]⁻ 839.2971 (calculated for C₃₉H₅₁O₂₀ 839.2972).

Author Contributions

S. Şimsek performed the chromatographic and spectral analyses. The antiproliferative assay was performed by A.Aydın. H. Akşit

and E. Köksal supervised. All authors contributed to the final manuscript and discussed the result.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: α -glucosidase inhibitory · antioxidant activity · antiproliferative activity · secondary metabolite · *Verbascum leiocarpum*

- [1] M. F. Cordeiro, T. R. S. Nunes, F. G. Bezerra, P. K. M. Damasco, W. A. V. Silva, M. R. A. Ferreira, O. M. C. Magalhães, L. A. L. Soares, I. M. F. Cavalcanti, M. Pitta, *Braz. J. Biol.* **2021**, 82–94.
- [2] K. F. Rodrigues, T. C. Bitencourt, J. G. Núñez, H. O. Garcia, B. Buhl, G. L. Padilha, E. M. Ethur, L. Hoehne, A. N. Bruno, E. M. Freitas, *Braz. J. Biol.* **2022**, 84, e255529–255536.
- [3] M. J. Balunas, A. D. Kinghorn, *Life Sci.* **2005**, 78, 431–441.
- [4] A. Onder, M. N. Izgi, A. S. Cinar, G. Zengin, M. A. Yilmaz, *S. Afr. J. Bot.* **2022**, 150, 313–322.
- [5] Ç. Şenkul, S. Kaya, *Turkish Geog. Rev.* **2017**, 69, 109–120.
- [6] F. A. Karavelioğulları, E. Yüce, B. Başer, *Phytotaxa* **2014**, 181, 47–53.
- [7] N. Y. Iskender, N. Yayli, N. Yildirim, T. B. Cansu, S. Terzioğlu, *J. Oleo Sci.* **2009**, 58, 117–121.
- [8] H. I. M. Amin, F. H. S. Hussain, G. Gilardoni, Z. M. Thu, M. Clericuzio, G. Vidari, *Plants* **2020**, 9, 1066–1085.
- [9] Y. L. Zhao, S. F. Wang, Y. Li, Q. X. He, K. C. Liu, Y. P. Yang, X. L. Li, *Arch. Pharmacol. Res.* **2011**, 34, 703–707.
- [10] S. Demirci, A. Doğan, Y. Demirci, F. Şahin, *Int. J. Appl. Res. Nat. Prod.* **2014**, 7, 37–44.
- [11] Z. Akdemir, Ç. Kahraman, I. I. Tatli, E. K. Akkol, I. Süntar, H. Keles, *J. Ethnopharmacol.* **2011**, 136, 436–443.
- [12] E. Kozan, I. T. Çankaya, C. Kahraman, E. K. Akkol, Z. Akdemir, *Exp. Parasitol.* **2011**, 129, 211–214.
- [13] A. Rezaie, M. Ebrahimi, E. Issabeagloo, M. Kumar, M. Nazari, S. Rezaie, S. Zakhireh, *Adv. Bio. Res.* **2012**, 3, 84–89.
- [14] R. Karamian, F. Ghasemlou, *J. Med. Plant Res.* **2013**, 2, 43–51.
- [15] E. K. Akkol, I. I. Tatli, Z. S. Akdemir, *Z. Naturforsch. C* **2007**, 62, 813–820.
- [16] H. A. E. H. El Gizawy, M. A. Hussein, E. Abdel-Sattar, *Pharm. Biol.* **2019**, 57, 485–497.
- [17] A. Gökmen, N. Kúsz, N. Karaca, F. Demirci, J. Hohmann, H. Kirmizibekmez, *Nat. Prod. Res.* **2021**, 35, 5294–5298.
- [18] H. Y. Gondal, R. Zamir, M. Nisar, M. I. Choudhary, *Nat. Prod. J.* **2020**, 10, 158–162.
- [19] I. Tatli, Z. Ş. Akdemir, E. Bedir, I. A. Khan, *Turk. J. Chem.* **2003**, 27, 765–772.
- [20] A. Pianaro, J. P. Pinto, D. T. Ferreira, N. K. Ishikawa, R. Braz-Filho, *Semina: Cienc. Agrar.* **2007**, 28, 251–255.
- [21] D. R. Encarnacion, L. C. Nogueiras, V. H. A. Salinas, U. Anthoni, P. H. Nielsen, C. Christophersen, H. Wang, X. Yao, J. P. Tuchagues, M. Ögren, *Acta Chem. Scand.* **1999**, 53, 375–377.
- [22] E. R. Woo, M. S. Piao, *Arch. Pharmacol. Res.* **2004**, 27, 173–176.
- [23] T. Sarg, O. Salama, M. El-Domiaty, M. Bishr, S. E. S. S. Mansour, E. Weight, *Alexandria J. Pharm. Sci.* **1991**, 1, 82–86.
- [24] R. Owen, R. Haubner, W. Mier, A. Giacosa, W. Hull, B. Spiegelhalder, H. Bartsch, *Food Chem. Toxicol.* **2003**, 41, 703–717.
- [25] N. Rønsted, E. Göbel, H. Franzyk, S. R. Jensen, C. E. Olsen, *Phytochemistry* **2000**, 55, 337–348.
- [26] Z. Boghrati, M. Naseri, M. Rezaie, N. Pham, R. J. Quinn, Z. Tayarani-Najaran, M. Iranshahi, *Iran. J. Basic Med. Sci.* **2016**, 19, 804–811.
- [27] Z. Güvenalp, H. Özbek, T. Ünsalar, C. Kazaz, L. Demirezer, *Turk. J. Chem.* **2006**, 30, 391–400.
- [28] J. Schlauer, J. Budzianowski, K. Kulczanka, L. Ratajczak, *Acta Soc. Bot. Pol.* **2004**, 73, 9–15.
- [29] İ. Calis, P. Akbay, A. Kuruuzum, F. Yalcin, P. Sahin, G. F. Pauli, *Pharmazie* **1999**, 54, 926–930.
- [30] F. Pardo, F. Perich, R. Torres, F. D. Monache, *J. Chem. Ecol.* **1998**, 24, 645–653.
- [31] L. Gao, X. Xu, H. Nan, J. Yang, G. Sun, H. Wu, M. Zhong, *J. Med. Plants Res.* **2012**, 6, 929–931.
- [32] H. Akşit, *Rec. Nat. Prod.* **2023**, 17, 501–508.
- [33] A. Hamed, A. Pasdaran, A. Pasdaran, *Bioorg. Chem.* **2020**, 99, 103776–103786.
- [34] Q. Liu, H. J. Hu, P. F. Li, Y. B. Yang, L. H. Wu, G. X. Chou, Z. T. Wang, *Phytochemistry* **2014**, 103, 196–202.
- [35] T. C. Akman, S. Simsek, Ö. Kayir, Z. Aksit, H. Aksit, N. Genc, *Chem. Biodiversity* **2023**, 20, e202201189–202201199.
- [36] H. Akşit, S. Gözcü, A. Altay, *Phytochemistry* **2023**, 205, 113484–113491.
- [37] Z. Güvenalp, H. Özbek, B. Dursunoğlu, H. Yuca, S. Gözcü, Y. M. Çil, C. Kazaz, K. Kara, Ö. L. Demirezer, *Med. Chem. Res.* **2017**, 26, 3209–3215.
- [38] I. E. Mohamed, E. El Bushra, M. I. Choudhary, S. N. Khan, *Rec. Nat. Prod.* **2009**, 3, 198–203.
- [39] H. Özbek, H. Yuca, S. Gözcü, B. Dursunoğlu, N. Özenver, Z. Güvenalp, C. Kazaz, M. Önal, L. Ö. Demirezer, *FABAD J. Pharm. Sci.* **2019**, 44, 127–132.
- [40] M. S. Blois, *Nature* **1958**, 181, 1199–1200.
- [41] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, *Free Radic. Biol.* **1999**, 26, 1231–1237.
- [42] M. Oyaizu, *J. Nutr.* **1986**, 44, 307–315.
- [43] J. Asghari, C. K. Touli, M. Mazaheritehrani, *J. Med. Plants Res.* **2012**, 6, 4475–4480.
- [44] S. Karakaya, S. Gözcü, Z. Güvenalp, H. Özbek, H. Yuca, B. Dursunoğlu, C. Kazaz, C. S. Kiliç, *Pharm. Biol.* **2018**, 56, 18–24.

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