INTRODUCTION

Verbascum is a widespread genus of the family Scrophulariaceae, which comprises more than 300 species in the world's flora[1]. This genus is represented by 233 species, 196 of which are endemic in Turkish Flora[2-4]. Infusions prepared by leaves and flowers of *Verbascum* species have been used as an expectorant and mucolytic[5], wound healer[6], for the treatment of hemorrhoid and rheumatism[7] in folk medicine. Turker and Camper[8] showed that *K. pneumoniae* and *S. aureus* showed sensitivity to the Mullein (*V. thapsus*), which may explain why Mullein is used in folk medicine to treat respiratory disorders (caused by *K. pneumoniae* and *S. aureus*) and urinary tract infections (caused by *K. pneumoniae*). Antibacterial and antifungal activities of *Verbascum* L. species have been previously reviewed and, it has been revealed the activity of the genus against several bacteria and fungi[9]. The antimicrobial activity of *V. mucronatum* has also been determined by disc diffusion method by our research group[10]. In addition, *V. mucronatum* Lam. has been used as an hemostatic in Turkish traditional medicine[11].

Previous investigations on Turkish *Verbascum* L. species by our research group led to the isolation and characterization of a number of secondary metabolites such as iridoids, monoterpene glucosides, saponins, phenylethanoids, neolignans and flavonoid glycosides[12-16]. As a part of our ongoing studies on the secondary metabolites of *Verbascum* L. species, we have now investigated the methanolic extract of the flowery parts of *V. mucronatum*, and isolated four iridoids; ajugol (**1**), aucubin (**2**), lasianthoside I (**3**), catalpol (**4**), two saponins; ilwensisaponin C (**5**) and A (**6**), along with a phenylethanoid glycoside, verbascoside (=acteoside) (**7**) by means of various chromatographic techniques (Figure). The current paper deals with the isolation, structure elucidation of the compounds (**1**-**7**) from the title plant and evaluation of their antimicrobial activities.

MATERIAL AND METHOD

General Experimental Procedures

The UV spectra (λ_{max}) were recorded on a Agilent 8453 spectrophotometer. The IR spectra (ν_{max}) were determined on a Perkin Elmer 2000 FT-IR spectrophotometer. The 1D and 2D NMR spectra were obtained on a Bruker Avance DRX 500 and 400 FT spectrometer operating at 500 and 400 MHz for ^{1}H NMR, and 125 and 100 MHz

for ^{13}C NMR. For the ^{13}C NMR spectra, multiplicities were determined by a distortionless enhancement by a polarization transfer (DEPT) experiment. LC-ESIMS data were obtained using a Bruker BioApex FT-MS instrument in the ESI mode. Reversed-phase material (C-18, LiChroprep 25-40 µm) and polyamide were used for vacuum liquid chromatography (VLC), reversed-phase material (C-18, LiChroprep 25-40 µm) was used for middle pressure liquid chromatography (MPLC), Si gel (230-400 mesh) (Merck) was used for column chromatography (CC). Pre-coated silica gel 60 F_{254} aluminum sheets (Merck) were used for thin layer chromatography (TLC); developing systems, $CHCl_3$-MeOH-H_2O (61:32:7 and 80:20:2). Plates were examined by UV fluorescence and sprayed with 1% vanillin in concentrated H_2SO_4, followed by heating at 105 ºC for 1-2 min.

Plant Material

V. mucronatum Lam. was collected from Aksaray, 17th. km of the road of Aksaray-Ulukışla in July 2007. A voucher specimen has been deposited in the Herbarium of the Faculty of Science, Gazi University, Ankara, Turkey (GAZI 10097). Flowery parts of the plant dried on air and shade were used in phytochemical studies.

Extraction and Isolation

Air-dried and powdered flowery parts of the plant (586.2 g) were extracted with MeOH (3 x 2,5 L). The MeOH extract was evaporated to dryness in vacuo to yield 70.4 g of crude extract, then MeOH extract was dissolved with 100 mL distilled water and partitioned in $CHCl_3$ (2 x 100 mL). H_2O and $CHCl_3$ phases were evaporated to dryness in vacuo to yield 65.8 g H_2O and 3.6 g $CHCl_3$ extracts. H_2O phase was fractionated by CC on polyamide (150 g) using H_2O-MeOH (100:0→0:100) (each 500 mL) respectively, to yield 6 fractions (Frs. A-F). Fraction D (4.9 g), eluted with 75% methanol, was subjected to VLC using reversed-phase material (C-18, LiChroprep 25-40 µm, 150 g), using MeOH-H_2O mixtures (0-100%) to give, catalpol (**4**) (62.1 mg), aucubin (**2**) (139.3 mg), ajugol (**1**) (48.6 mg), Fr. D3 (1.19 g) and Fr. D4 (625.3 mg). Frs. D3 and D4 were rechromatographed. Fr. D3 was applied to MPLC using reversed-phase material (C-18, LiChroprep 25-40 µm) using MeOH-H_2O mixtures (100:0→30-70) to yield ilwensisaponin C (**5**) (14.7 mg), ilwensisaponin A (**6**) (51.5 mg) and lasianthoside I (**3**) (6.7 mg). Fr. D4 was rechromatographed on a silica gel column (55 mg) and eluted $CHCl_3$-MeOH (70:30→60:40) mixtures to give verbascoside (=acteoside) (**7**) (14.8 mg).

Antimicrobial Activity-Broth Microdilution Method

Antibacterial and antifungal activities were determined using the broth microdilution test method as recommended by Clinical and Laboratory Standards Institute[17,18]. Plant extracts were tested against four bacteria including two Gram positive (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212) and two Gram negative microorganisms (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) as well as for antifungal activities against three yeasts (*Candida albicans* ATCC 90028, *Candida krusei* ATCC 6258, *Candida parapsilosis* ATCC 90018). Antibacterial activity test was performed in Mueller-Hinton broth (MHB, Difco Laboratories, Detroit, MI, USA); for antifungal test, RPMI- 1640 medium with L-glutamine (ICN-Flow, Aurora, OH, USA), buffered with MOPS buffer (ICN-Flow, Aurora, OH, USA) was used. The inoculum densities were approximately $5x10^5$ cfu/mL and $0.5\text{-}2.5x10^3$ cfu/mL for bacteria and fungi, respectively.

Each plant extract was dissolved in 2.44 mL DMSO. Final two-fold concentrations were prepared in the wells of the microtiter plates, between 1024-1 µg/mL. Ampicillin and fluconazole were used as reference antibiotics for bacteria and fungi, respectively (64-0.0625 µg/mL). Microtiter plates were incubated at 35°C for 18-24 h for bacteria and 48 h for fungi. After the incubation period, minimum inhibitory concentration (MIC) values were defined as the lowest concentration of the extracts that inhibits the visible growth of the microorganisms.

RESULTS

Ajugol (1): UV λ_{max} (MeOH) 220 nm, IR (KBr) ν_{max} 3410 (OH), 1660 (C=C) cm^{-1}, Pozitif iyon LC-ESIMS m/z 371 $[M+Na]^+$ (calc. for $C_{15}H_{24}O_9$), ^1H NMR **(**400 MHz, DMSO-*d*$_6$) of **1**: δ_H 6.10 (1H, dd, *J*=6/1.6 Hz, H-3), 5.29 (1H, d, *J*=2 Hz, H-1), 4.78 (1H, dd, *J*=6/2.8 Hz, H-4), 4.43 (1H, d, *J*=7.6 Hz, H-1'), 3.71 (1H, d, *J*=2.8 Hz, H-6), 3.71-3.65 (2H, * , H-6'), 3.05-2.93 (1H, * , H-2', H-3', H-4', H-5'), 2.47 (1H, m, H-5), 2.32 (1H, t, *J*=10 Hz, H-9), 1.84 (1H, dd, *J*=12.8/6.0 Hz, H-7b), 1.63 (1H, dd, *J*=13.2/6.0 Hz, H-7a), 1.13 (3H, s, H-10) and ^{13}C NMR (100 MHz, DMSO-*d*6) (see Table 1).

Aucubin (2): UV λ_{max} (MeOH) 205 nm, (KBr) ν_{max} 3275 (OH), 1650 (C=C) cm^{-1}, Pozitif iyon LC-ESIMS m/z 369 $[M+Na]^+$ (calc. for $C_{15}H_{22}O_9$), ^1H NMR **(**400 MHz, DMSO-*d*$_6$) of **2**: δ_H 6.30 (1H, dd, *J*=4.8/1.6 Hz, H-3), 5.65 (1H, bs, H-7) 5.01 (1H, d, *J*=4.8 Hz, H-4), 4.95 (1H, d, *J*=5.6 Hz, H-1), 4.85 (1H, d, *J*=7.7 Hz, H-1'), 4.40, (1H, d, *J*=6.4 Hz, H-6), 4.14 (1H, dd, *J*=12.4/4.0 Hz, H-10b), 3.96 (1H, dd, *J*=12.4/4.0 Hz, H-10a), 3.66 (1H, dd, *J*=12.8/4.8 Hz, H-6'a), 3.42 (1H, dd, *J*=12.0/4.8 Hz, H-6'b), 3.16 (1H, m, H-3'), 3.11 (1H, m, H-4'), 3.04 (1H, m, H-5'), 3.00 (1H, m, H-2'), 2.72 (1H, t, *J*=7.2 Hz, H-9), 2.50 (1H, m, H-5), and ^{13}C NMR (100 MHz, DMSO-*d*6) (see Table 1).

Lasianthoside I (3): UV λ_{max} (MeOH) 216, 277 nm, IR (KBr) ν_{max} 3405 (OH), 1704 (C=O), 1655 (C=C), 1508, 1451 (aromatic ring) cm^{-1}, Pozitif iyon LC-ESIMS m/z 611 $[M+Na]^+$ (calc. for $C_{30}H_{38}O_{15}$), ^1H NMR **(**400 MHz, DMSO-*d*$_6$) of **3:** δ_H 6.37 (1H, dd, *J*=4.8/1.2 Hz, H-3), 5.26 (1H, d, *J*=4.4 Hz, H-4), 5.10 (1H, d, *J*=4.0 Hz, H-1), 4.91 (1H, d, *J*=7.6 Hz, H-1'), 4.18 (1H, d, *J*=6.0 Hz, H-10b), 3.86 (1H, d, *J*=4 Hz, H-6'b), 3.78 (1H, t, *J*=6.8 Hz, H-6), 3.66 (1H, * , H-10a), 3.64 (1H, dd, *J*=10.8/6.4 Hz, H-6'a), 3.35 (1H, s, H-7), 2.31 (1H, t, *J*=7.6 Hz, H-9), 3.13-3.19 (1H, * , H-3', H-4', H-5'), 3.02 (1H, dd, *J*=10/6.4 Hz, H-2'), 2.12 (1H, m, H-5) and ^{13}C NMR (125 MHz, DMSO-*d*6) (see Table 1).

Catalpol (4): UV λ_{max} (MeOH) nm 208 nm, IR (KBr) ν_{max} 3450 (OH), 1670 (C=C) cm^{-1}, Pozitif iyon LC-ESIMS m/z 385 $[M+Na]^+$ (calc. for $C_{15}H_{22}O_{10}$), ^1H NMR **(**400 MHz, DMSO-*d*$_6$) of **4**: δ_H 6.37 (1H, dd, *J*= 4.8/1.2 Hz, H-3), 5.26 (1H, d, *J*=4.4 Hz, H-4), 5.10 (1H, d, *J*=4.0 Hz, H-1), 4.91, (1H, d, *J*=7.6 Hz, H-1'), 4.18 (1H, d, *J*=6.0 Hz, H-10b), 3.86 (1H, d, *J*=4 Hz H-6'b), 3.78 (1H, t, *J*=6.8 Hz, H-6), 3.66 (1H, * , 10a), 3.64

(1H, dd, *J*=10.8/6.4 Hz, H-6'a), 3.35 (1H, s, H-7), 3.13-3.19 (* , H-3', H-4', H-5'), 3.02 (1H, dd, *J*=10/6.4 Hz, H-2'), 2.31 (1H, t, *J*=7.6 Hz, H-9), 2.12 (1H, m, H-5) and ^{13}C NMR (100 MHz, DMSO-*d*6) (see Table 1).

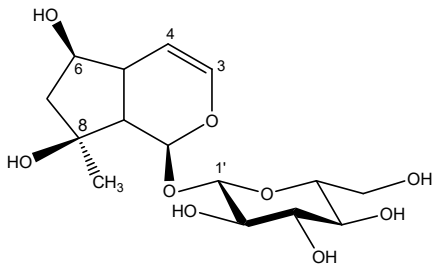
Ilwensisaponin C (5): UV λ_{max} (MeOH) 205 nm, IR (KBr) ν_{max} 3400 (OH), 1665 (C=C) cm^{-1}, Pozitif iyon LC-ESIMS m/z 1127 $[M+Na]^+$ (calc. for $C_{55}H_{92}O_{22}$), ^{1}H NMR **(**400 MHz, Pyridine) of **5**: δ_H 5.78 (1H, bs, H-1'''), 5.54 (1H, d, *J*=7.0 Hz, H-1''''), 5.46 (1H, bs, H-12), 5.21 (1H, d, *J*=7.0 Hz, H-1''), 4.91 (1H, d, *J*=6.6 Hz, H-1'), 4.35 (1H, * , H-2'), 4.33 (1H, * , H-23b), 4.10 (1H, * , H-2''''), 4.10 (1H, * , H-3), 3.89 (1H, * , H-2'), 3.82 (1H, * , H-11), 3.81 (1H, d, *J*=11.7 Hz, H-28b), 3.69 (1H, d, *J*=8.3 Hz, H-23a), 3.57 (1H, d, *J*=10.2 Hz, H-28a), 1.68 (3H, d, *J*=5.5 Hz, H-6'''), 1.35 (3H, d, *J*=4.8 Hz, H-6'), 1.30 (3H, s, H-27), 1.08 (3H, s, H-24), 1.07 (3H, s, H-25), 0.96 (3H, s, H-26), 0.95 (3H, s, H-30), 0.88 (3H, s, H-29), CH_3O: 3.21 (3H, s) and ^{13}C NMR (125 MHz, pyridine) (see Table 2).

Ilwensisaponin A (6): UV λ_{max} (MeOH) 206 nm, IR (KBr) ν_{max} 3434 (OH), 1645 (C=C) cm^{-1}, Pozitif iyon LC-ESIMS m/z 1095 $[M+Na]^+$ (calc. for $C_{54}H_{88}O_{21}$), ^{1}H NMR **(**500 MHz, Pyridine) of **6**: δ_H 5.94 (1H, d, *J*=10.4 Hz, H-11), 5.77 (1H, d, *J*=1.5 Hz, H-1'''), 5.53 (1H, * , H-12), 5.20 (1H, d, *J*=7.6 Hz, H-1''), 5.53 (1H, d, *J*=7.9 Hz, H-1''''), 4.91 (1H, d, *J*=7.7 Hz, H-1'), 4.58 (1H, * , H-2'''), 4.34 (1H, * , H-23b), 4.25 (1H, * , H-2'), 4.11 (1H, * , H-3), 4.05 (1H, * , H-2''''), 3.90 (1H, * , H-2''), 3.72 (1H, * , H-28b), 3.70 (1H, * , H-23a), 3.33 (1H, d *J*=6.2 Hz, H-28a), 1.68 (1H, d, *J*=6.1 Hz, H-6'''), 1.38 (3H, bs, H-6'), 1.31 (3H, s, H-26), 1.04 (3H, s, H-24), 0.98 (3H, s, H-27), 0.96 (3H, s, H-25), 0.87 (3H, s, H-29), 0.82 (3H, s, H-30) and ^{13}C NMR (125 MHz, CD_3OD) (see Table 2).

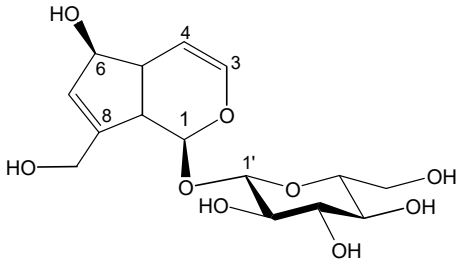
Verbascoside (Acteoside) (7): UV λ_{max} (MeOH) 220, 332 nm, IR (KBr) ν_{max} 3392 (OH), 1699 (C=O), 1631 (C=C), 1604, 1525 (aromatic ring) cm^{-1}, Pozitif iyon LC-ESIMS m/z 647 $[M+Na]^+$ (calc. for $C_{29}H_{36}O_{15}$), ^{1}H NMR **(**500 MHz, DMSO-*d*$_6$) of **7**: δ_H 7.48 (1H, d, *J*=15.8 Hz, H-*β*'), 7.04 (1H, s, H-2'''), 6.97 (1H, d, *J*=7.5 Hz, H-6'''), 6.79 (1H, d, *J*=7.7 Hz, H-5'''), 6.67 (1H, bs, H-2), 6.67 (1H, bs, H-5), 6.52 (1H, d, *J*=7.5 Hz, H-6), 6.20 (1H, d, *J*=15.8 Hz, H-*α*'), 5.07 (1H, bs, H-1''), 4.75 (1H, t, *J*=9.4 Hz, H-4'), 4.37 (1H, d, *J*=7.7 Hz, H-1'), 3.72 (1H, * , H-2''), 3.91, (1H, m, H-α_b), 3.67, (1H, m, H-α_a), 2.73 (2H, s, H-*β*), 3.68 (1H, * , H-3'), 3.45-3.70 (2H, * , H-6'), 3.45 (1H, * , H-5'),

3.36 (1H, * , H-5''), 3.35 (1H, * , H-3''), 3.26 (1H, t, *J*=8.3 Hz, H-2'), 3.15 (1H, * , H-4''), 1.00 (3H, d, *J*=5.8 Hz, H-6'') and ^{13}C NMR (125 MHz, $CDCl_3$) (see Table 3).

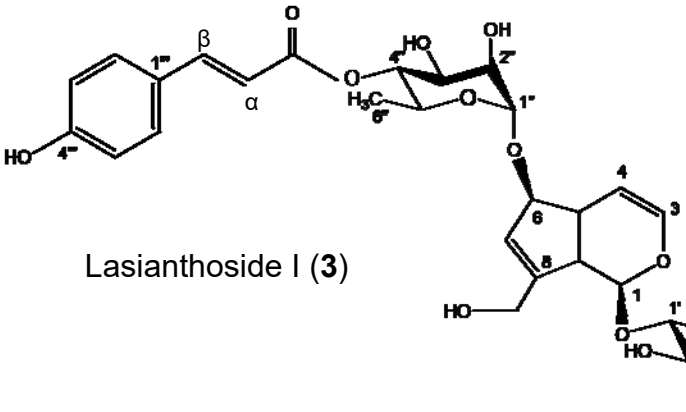
* (*overlapped*)



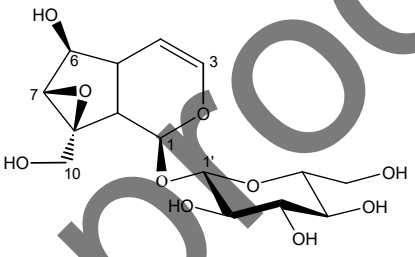
Ajugol (**1**)



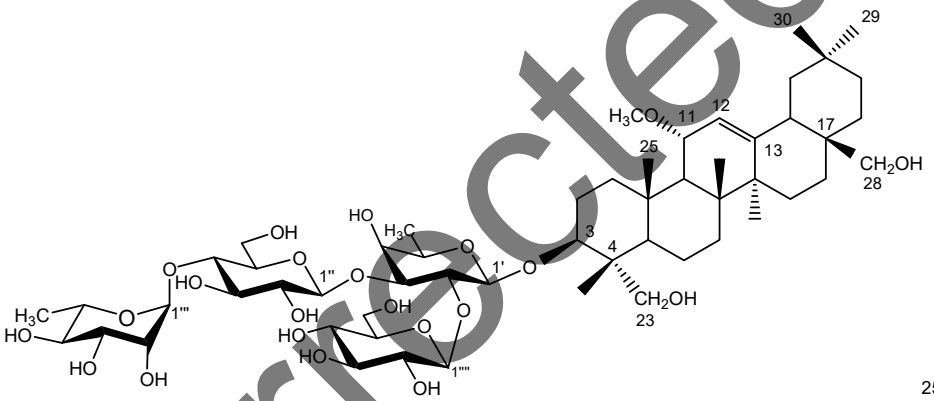
Aucubin (**2**)



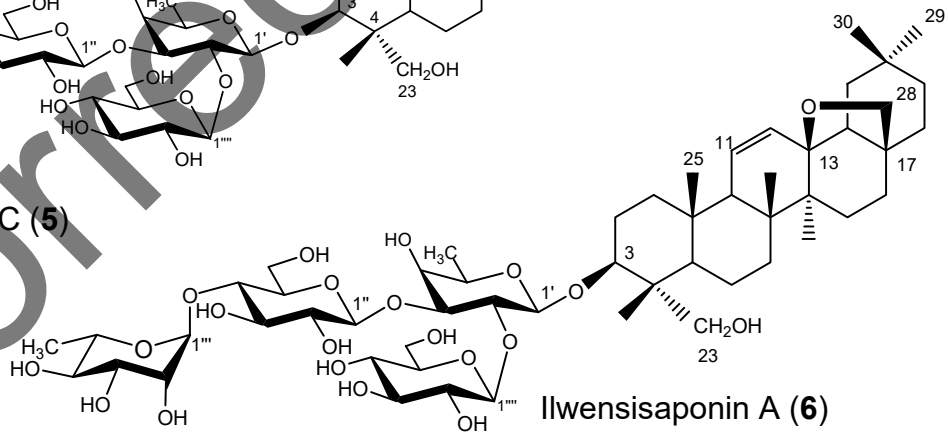
Lasianthoside I (**3**)



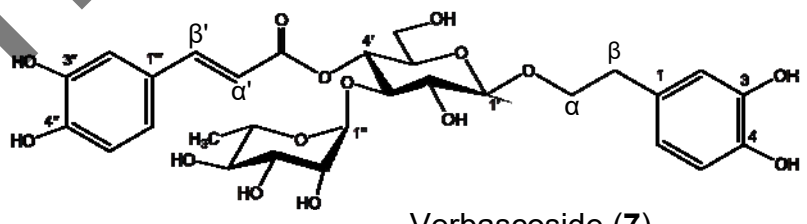
Catalpol (**4**)



Ilwensisaponin C (**5**)



Ilwensisaponin A (**6**)



Verbascoside (**7**)

Figure. Isolated secondary metabolites from *V.mucronatum* Lam.

Table 1. ^{13}C NMR (DMSO-d_6) data of compounds of **1**, **2**, **3** and **4**

C/H Atom	**2** (100 MHz**)** δc (ppm)	**3** (125 MHz) δc (ppm)	**4** (100 MHz**)** δc (ppm)	**1** (100MHz) δc (ppm)
Aglycone				
1	95.9	96.0	93.8	92.1
3	140.6	141.1	140.7	139.3
4	105.6	104.8	103.8	105.7
5	45.2	42.8	37.8	40.7
6	81.1	87.5	77.8	77.8
7	129.8	125.6	61.2	50.6
8	146.8	149.4	65.3	77.7
9	47.0	47.3	42.6	50.5
10	60.1	59.9	59.5	25.7
Glc at C-1				
1'	98.7	100.1	98.3	98.1
2'	74.0	73.9	73.8	73.8
3'	77.3	77.2	76.8	76.1
4'	70.7	70.7	70.6	70.7
5'	77.8	77.6	77.6	77.4
6'	61.7	61.6	61.7	61.7

Compound 3: Rha at C-6, 98.6 (C-1''), 74.3 (C4''), 71.4 (C-2''), 68.8 (C-3''), 67.0 (5''), 18.0 (C-6''); **Acyl moiety**, 166.8 (C=O), 161.0 (C-4'''), 145.2 (C-*β*), 133.0 (1'''), 130.7 (C-2'''), 130.7 (C-6'''), 116.3 (C-3'''), 116.3 (C-5'''), 115.4 (C-*α*).

Table 2. ^{13}C NMR (125 MHz, Pyridine-d5/**5**, CD_3OD/**6**) data of compounds **5** and **6**

	5	**6**		**5**	**6**
C/H Atom	δc (ppm)	δc (ppm)	C/ Atom	δc (ppm)	δc (ppm)
Aglycone			Sugar units		
1	40.2	38.0	Fuc at C-3		
2	22.9	25.6	1′	104.2	104.7
3	83.0	84.0	2′	77.0	77.1
4	44.1	45.9	3′	85.0	85.7
5	48.1	46.0	4′	72.2	72.2
6	18.5	18.0	5′	70.6	70.7
7	31.9	31.0	6′	17.3	17.0
8	37.6	42.6	Glc at Fuc C-3′		
9	52.8	54.1	1″	105.1	105.1
10	35.8	37.0	2″	75.6	75.4
11	76.2	132.9	3″	77.8	76.1
12	122.6	131.9	4″	78.4	79.3
13	148.1	86.9	5″	77.2	76.4
14	43.6	44.1	6″	61.4	63.5
15	26.7	26.0	Rha at Glc C-4″		
16	26.4	26.5	1‴	102.8	102.9
17	42.2	40.0	2‴	72.8	72.7
18	42.5	52.8	3‴	72.6	71.3
19	47.1	38.3	4‴	74.0	73.8
20	31.4	31.0	5‴	70.5	70.7
21	33.3	34.0	6‴	18.5	18.5
22	34.8	32.0	Glc at Fuc C-2′		
23	64.8	64.5	1⁗	104.0	103.5
24	13.4	12.6	2⁗	76.2	75.4
25	18.0	19.0	3⁗	78.8	76.8
26	18.7	22.0	4⁗	72.2	73.5
27	26.4	20.0	5⁗	76.5	78.3
28	68.9	78.3	6⁗	63.3	61.8
29	33.5	34.0			
30	24.0	24.0			
OCH_3	54.1	-			

Table 3. ^{13}C NMR (125 MHz, $CDCl_3$) Data of compoud **7**

	7		
C /Atom	δ_C (ppm)	C/ Atom	
Aglycone		Rha at Glc C-3′	
1	131.5	1″	103.1
2	117.2	2″	72.3
3	146.7	3″	72.1
4	144.3	4″	73.9
5	116.7	5″	70.5
6	120.5	6″	18.9
α	71.4	Acyl moiety	
β	35.9	1‴	127.7
		2‴	115.6
Glc		3‴	146.9
1′	104.3	4‴	149.9
2′	76.3	5‴	116.4
3′	81.7	6‴	122.2
4′	70.7	α'	114.7
5′	76.1	β'	148.1
6′	62.7	C=O	168.3

Table 4. Minimum Inhibitory Concentrations (MIC - µg/mL) of the methanolic extract and the secondary metabolites

	Bacteria				Fungi		
	S. aureus	*E. faecalis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*C. krusei*	*C. parapsilosis*
	ATCC 29213	ATCC 29212	ATCC 25922	ATCC 27853	ATCC 90028	ATCC 6258	ATCC 22019
V. mucronatum-MeOH extract	256	128	256	256	256	128	128
Ajugol	128	256	128	128	64	128	64
Aucubin	256	512	512	256	128	256	256
Lasianthoside I	>512	512	512	512	256	512	256
Catalpol	256	512	512	256	256	256	256
Ilwensisaponin C	>512	>512	512	512	256	512	256
Ilwensisaponin A	256	>512	>512	512	64	64	128
Verbascoside	256	512	512	256	256	256	256
Ampicillin	1	8	2	-	-	-	-
Fluconazole	-	-	-	-	1	64	8

Methanolic extract of the flowery part of *V. mucronatum* and isolated compounds possessed moderate antimicrobial activity especially against fungi. Iridoid glycoside ajugol was found to be most active compound against to *C. albicans* and *C. parapsilosis* with the 64 µg/mL MIC value, as well as ilwensisaponin A inhibited *C. albicans* and *C. krusei* with the same MIC value as those of the ajugol. These active compounds were found to be effective against to fungi much more than *V. mucronatum* extract.

DISCUSSION

Compound **1** was isolated as a white amorphous powder with the molecular formula $C_{15}H_{24}O_9$ (LC-ESIMS *m/z* 371 $[M+Na]^+$). An iridoid enolether system (220 nm) in UV spectrum; hydroxyl group (3410 cm^{-1}) and a double bond (1660 cm^{-1}) absorption bands in IR spectra were observed. Compound **1** was identified as ajugol comparing ^1H and ^{13}C NMR spectra with those of ajugol[19].

Compound **2** (See Figure) was isolated as white amorphous powder with the molecular formula $C_{15}H_{22}O_9$ (LC-ESIMS *m/z* 369 $[M+Na]^+$). An iridoid enolether system (205 nm) in UV spectrum; hydroxyl group (3275 cm^{-1}) and a double bond (1650 cm^{-1}) absorption bands in IR spectra were observed. Compound **2** was identified as aucubin comparing ^1H and ^{13}C NMR spectra with those of aucubin[20,21].

Compound **3** (see Figure) was isolated as a white amorphous powder with the molecular formula $C_{30}H_{38}O_{15}$ (LC-ESIMS *m/z* 661 $[M+Na]^+$). Presence of an iridoid enolether system (216 nm) and an aromatic acid (277 nm) moiety in UV spectrum and absorption bands for a hydroxyl group (3405 cm^{-1}), a conjugated ester carbonyl (1704 cm^{-1}), a double bond (1655 cm^{-1}) and an aromatic ring (1451 cm^{-1}, 1508 cm^{-1}) in IR spectra were observed. The ^1H and ^{13}C NMR spectra of **3** were similar to those of lasianthoside I. Based on this evidence, compound **3** was identified as lasianthoside I[22].

Compound **4** (see Figure) was isolated as a white amorphous powder with the molecular formula $C_{15}H_{22}O_{10}$ (LC-ESIMS *m/z* 385 $[M+Na]^+$). Its UV spectrum supported the presence of an iridoid enolether system (208 nm) and absorption bands were for a hydroxyl group (3450 cm^{-1}) and a double bond (1670 cm^{-1}) in IR spectra were observed. The ^1H and ^{13}C NMR spectra of **4** were similar to those of catalpol. Thus, compound **4** was identified to be catalpol[23].

Compounds **5** and **6** (see Figure) were obtained as amorphous compounds with the molecular weights 1104 {LC-ESIMS: *m/z* 1127 ($[M+Na]^+$)}, and 1072 {LC-ESIMS: *m/z* 1095 ($[M+Na]^+$)}, as calculated for $C_{55}H_{92}O_{22}$ and $C_{54}H_{88}O_{21}$, respectively.

In their IR spectra, the observed absorbances were consistent with the presence of olefinic double bonds. The ^1H and ^{13}C NMR data of **5** and **6** suggested that they had similar structures, possessing the same sugar moieties but differing in their aglycones.

In the ^{1}H NMR spectrum of **5**, characteristic resonances for anomeric protons were observed at δ_H 4.91 (*d*, *J*= 6.6 Hz), 5.21 (*d*, *J*= 7.0 Hz), 5.54 (*d*, *J*= 7.0 Hz), 5.78 (*bs*), and, in the ^{13}C NMR spectrum, anomeric carbons at δ_c 104.2 (*β*-D-fucopyranose), 105.1 (*β*-D-glucopyranose-inner), 104.0 (*β*-D-glucopyranose-terminal) and 102.8 (*α*-L-rhamnopyranose), as well as 2 proton signals at δ_H 1.35 (*d*, *J*= 4.8 Hz) and 1.68 (*d*, *J* = 5.5 Hz), arising from the secondary methyl groups in the sugar moieties. By means of HMBC correlations, the sequence of the saccharidic chain was determined as [α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→3)]-[*β*-D-glucopyranosyl-(1→2)]-*β*-D fucopyranoside.

The ^{1}H NMR of **5** showed 6 tertiary methyl signals at δ_H 0.88, 0.95, 0.96, 1.07, 1.08 and 1.30. The proton signal at δ_H 3.21 (3H) was attributed to methoxy protons, and δ_H 5.46 (*br s*) to the olefinic proton of the aglycone. It has been determined that the aglycone was an oleanane-Δ^{12} type confirmed by presence of δ_c 122.6 and 148.1 signals in the ^{13}C NMR spectrum. The assignment of the remaining NMR signals was achieved by means of ^{1}H-^{1}H COSY, HMQC and HMBC experiments.

The location of the methoxy group was determined by HMBC correlations between methoxy protons and C-11, whereas the chemical shift of C-11 (δ_c 76.2) was also evident. From the chemical shift of C-11 (δ_c 76.2) in **5**, it can be concluded that the methoxyl group has an *α*-configuration as reported for saikosaponin-b$_4$ [24] .The H-3 methine proton, H-23 and H-28 methylene protons showed downfield shifts due to hydroxy substitutions.

Consequently, the structure was elucidated to be 3-*O*-{[*α*-L-rhamnosyl-(1→4)-(*β*-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl]-(1→2)-*β*-D-fucopyranosyl-11-methoxy-olean-12-ene-3*β*,23,28-triol (= ilwensisaponin C)[25] .

Compound **6** was distinguished by the differences in aglycone parts from compound **5** in ^{1}H and ^{13}C NMR spectra.

The ^{1}H NMR of **6** showed 6 tertiary methyl signals at δ_H 0.82, 0.87, 0.96, 0.98, 1.04 and 1.31. The olefinic protons H-11 and H-12 were determined at 5.94 (*br d*, *J*= 10.4 Hz), δ_c 132.9 and δ_H 5.53 (*), δ_c 131.9, respectively. Thus, aglycone was identified as an oleanane-Δ^{11} type and no signals of methoxy group in ^{1}H and ^{13}C NMR spectra of compound **6** were observed compared to those of compound **5**.

Due to presence of an oxo-bridge between C-28 and C-13, chemical shift of C-28 methylene protons (δ_H 3.33 - 3.72) were appeared in the higher field in

comparison to those of C-23 hydroxylated methylene protons (δ_H 3.70 - 4.34). Based on this evidence, the aglycone of **6** was determined as 13*β*, 28-epoxyolean-11-ene-3*β*,23-diol[26] .

As a result, the structure of **6** was determined to be 3-*O*-{[α-L-rhamnosyl-(1→4)-(*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranosyl]-(1→2)-*β*-D-fucopyranosyl}-13*β*,28-epoxyolean-11-ene-3*β*,23-diol (=ilwensisaponin A[25]= mimengoside A[27]).

Compound **7** (see Figure) was obtained as an amorphous powder. Its structure was identified as verbascoside by comparing its ^{1}H and DEPT-^{13}C NMR data with previously published data and by direct comparison with the authentic sample on a TLC plate.

It has been reported that *Verbascum* L. species contain diverse iridoid glycosides such as ajugol[5,13], aucubin[28], lasianthoside I[22] and catalpol[23]; saponins such as ilwensisaponin C[13] and ilwensisaponin A[13]; phenylethanoid glycosides such as verbascoside[13]. Ilwensisaponin A previously found to be active against *Aspergillus fumigatus*[29] showed a moderate antifungal activity against fungi in our current study.

Conclusion

This paper is the first report of the presence of these compounds from *V. mucronatum* Lam. Our continuing studies will be of assistance in clarifying the chemotaxonomical classification of the genus *Verbascum* L. On the other hand, when the antimicrobial activity results were evaluated, higher activities of ajugol and ilwensisaponin A than the *V. mucronatum* extract suggest that more active compounds may be found in further phytochemical studies.

Acknowledgement

The authors would like to thank Prof. Dr. Hayri Duman, Gazi University, Faculty of Science, Department of Botany, Etiler, Ankara, Turkey, for authentification of the plant specimen.

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