Structure Elucidation and Antimicrobial Activities of Secondary Metabolites from the Flowery Parts of *Verbascum mucronatum* Lam.

*Verbascum mucronatum* Lam.’s flowery parts were used to obtain secondary metabolites and to evaluate their antimicrobial activity.

**Objectives:** To determine the secondary metabolites from *Verbascum mucronatum* Lam. and evaluate their antimicrobial activity.

**Materials and Methods:** Antimicrobial activities of the isolated metabolites were determined using broth microdilutions against the bacteria (*Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213) and fungi (*Candida albicans* ATCC 90028, *Candida krusei* ATCC 6258, *Candida parapsilosis* ATCC 90018).

**Results:** Four iridoid glycosides, ajugol (1), aucubin (2), lasianthoside I (3), catalpol (4), two triterpenic saponins, ilwensisaponin C (5), ilwensisaponin A (=mimengoside A) (6), and one phenylethanoid glycoside, verbascoside (=acteoside) (7) were isolated from the water soluble parts of the methanolic extract gained flowery parts of *V. mucronatum* Lam.

**Conclusion:** Within the obtained compounds, ajugol and ilwensisaponin A showed moderate antimicrobial activity, especially against fungi.

**Key words:** Scrophulariaceae, *Verbascum mucronatum* Lam., secondary metabolites, antimicrobial activity

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**INTRODUCTION**

*Verbascum* is a widespread genus of the family Scrophulariaceae, which comprises more than 300 species of the world’s flora. This genus is represented by 233 species, 196 of which are endemic in Turkish flora. Infusions prepared with the leaves and flowers of *Verbascum* species have been used as an expectorant and mucolytic wound healer for the treatment of hemorrhoids and rheumatism in folk medicine. Turker and Camper showed that *Klebsiella pneumoniae* and *Staphylococcus aureus* showed sensitivity to Mullein (*Verbascum 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 the activity of the genus against several bacteria and fungi has been revealed. The antimicrobial activity of *Verbascum mucronatum* has also been determined using disc diffusion tests by our research group. In addition, *V. mucronatum* Lam. has been used as a Hemostatic in Turkish traditional medicine.

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, monoterpenes, glucosides, saponins, phenylethanoids, neolignans, and flavonoid glycosides. 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 ilwensisaponin A (6), along with a phenylethanoid glycoside, verbascoside (=acteoside) (7) by means of various chromatographic techniques (Figure 1). The current paper deals with the isolation and structure elucidation of the compounds (1-7) from the title plant and the evaluation of their antimicrobial activities.

### MATERIALS AND METHODS

#### General experimental procedures

The ultraviolet (UV) spectra (λ<sub>max</sub>) were recorded on a Agilent 8453 spectrophotometer. The infrared (IR) spectra (ν<sub>max</sub>) were determined on a Perkin Elmer 2000 fourier transform (FT)-IR spectrophotometer. The 1D and 2D nuclear magnetic resonance (NMR) spectra were obtained on a Bruker Avance DRX 500 and 400 FT spectrometer operating at 500 and 400 MHz for 1H NMR, and 125 and 100 MHz for 13C NMR. For the 13C NMR spectra, multiplicities were determined using distortionless enhancement with a polarization transfer (DEPT) experiment. LC-ESIMS data were obtained using a Bruker BioApex FT-mass spectrometry 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), and Si gel (230-400 mesh) (Merck) was used for column chromatography (CC). Pre-coated silica gel 60 F<sub>254</sub> aluminum sheets (Merck) were used for thin-layer chromatography (TLC); developing systems, CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O (60:40:2) mixtures to give verbascoside (acteoside) (7) by means of various chromatographic techniques (Figure 1). The current paper deals with the isolation and structure elucidation of the compounds (1-7) from the title plant and the evaluation of their antimicrobial activities.

### Plant material

*V. mucronatum* Lam. was collected from Aksaray, 17 km from Aksaray to Ulukışla, in July 2007. A voucher specimen has been deposited in the Herbarium of the Faculty of Science, Gazi University, Ankara, Turkey (GAZI 10097). The flowery parts of the plant, which were air dried in the shade, were used in the phytochemical studies.

### Extraction and isolation

Air-dried and powdered flowery parts of the plant (586.2 g) were extracted with MeOH (3x2.5 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<sub>3</sub> (2x100 mL). H<sub>2</sub>O and CHCl<sub>3</sub> phases were evaporated to dryness in vacuo to yield 65.8 g H<sub>2</sub>O and 3.6 g CHCl<sub>3</sub> extracts. The H<sub>2</sub>O phase was fractionated using CC on polyamide (150 g) using H<sub>2</sub>O-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<sub>2</sub>O mixtures (0-100%) to give catalpol (4) (62.1 mg), aucubin (2) (139.3 mg), ajugol (1) (48.6 mg), Fr. D3 (119 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<sub>2</sub>O 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<sub>3</sub>-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 as recommended by Clinical and Laboratory Standards Institute. Plant extracts were tested against four bacteria including two Gram-positive (*S. 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). The 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 5×10<sup>6</sup> CFU/mL and 0.5-2.5×10<sup>3</sup> CFU/mL for bacteria and fungi, respectively.

Each plant extract was dissolved in 2.44 mL DMSO. Finally, 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<sub>max</sub> (MeOH) 220 nm, IR (KBr) ν<sub>max</sub> 3410 (OH), 1660 (C=O) cm<sup>-1</sup>. Positive ion LC-ESIMS m/z 371 [M+Na]<sup>+</sup> (calc. for C<sub>24</sub>H<sub>27</sub>O<sub>3</sub>Na, 1H NMR (400 MHz, DMSO-d<sub>6</sub>) of 1: δ<sub>H</sub> 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<sub>10</sub>=10 Hz, H-9), 1.84 (1H, J<sub>10</sub>=7.6 Hz, H-2), 1.26 (3H, "H-9'", H-8), 0.82 (3H, "H-10"), 0.72 (3H, "H-10'"").
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 13C NMR (100 MHz, DMSO-d6) (see Table 1).

**Aucubin (2):** UV \( \lambda_{\text{max}} \) (MeOH) 205 nm, (KBr) \( \nu_{\text{max}} \) 3275 (OH), 1650 (C=C) cm\(^{-1}\). Positive ion LC-ESIMS m/z 369 [M+Na\(^+\)] (calc. for C\(_{19}\)H\(_{21}\)O\(_{8}\)). \(^1\)H NMR (400 MHz, DMSO-d6) of 2: \( \delta \) 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 13C NMR (100 MHz, DMSO-d6) (see Table 1).

**Lasianthoside I (3):** UV \( \lambda_{\text{max}} \) (MeOH) 216, 277 nm, IR (KBr) \( \nu_{\text{max}} \) 3405 (OH), 1704 (C=O), 1655 (C=C), 1508, 1451 (aromatic ring) cm\(^{-1}\). Positive ion LC-ESIMS m/z 611 [M+Na\(^+\)] (calc. for C\(_{30}\)H\(_{38}\)O\(_{14}\)). \(^1\)H NMR (400 MHz, DMSO-d6) of 3: \( \delta \) 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=10.4/6 Hz, H-2'), 2.12 (1H, m, H-5), and 13C NMR (125 MHz, DMSO-d6) (see Table 1).

**Catalpol (4):** UV \( \lambda_{\text{max}} \) (MeOH) nm 208 nm, IR (KBr) \( \nu_{\text{max}} \) 3450 (OH), 1670 (C=C) cm\(^{-1}\). Positive ion LC-ESIMS m/z 385 [M+Na\(^+\)] (calc. for C\(_{30}\)H\(_{38}\)O\(_{14}\)). \(^1\)H NMR (400 MHz, DMSO-d6) of 4: \( \delta \) 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), 3.13-3.19 (1H, *-H-3', H-4', H-5'), 3.02 (1H, dd, J=10.4/6 Hz, H-2'), 2.31 (1H, t, J=7.6 Hz, H-9), 2.12 (1H, m, H-5), and 13C NMR (100 MHz, DMSO-d6) (see Table 1).

**Iwensissaponin C (5):** UV \( \lambda_{\text{max}} \) (MeOH) 205 nm, IR (KBr) \( \nu_{\text{max}} \) 3400 (OH), 1665 (C=C) cm\(^{-1}\). Positive ion LC-ESIMS m/z 1127 [M+Na\(^+\)] (calc. for C\(_{35}\)H\(_{34}\)O\(_{18}\)). \(^1\)H NMR (400 MHz, pyridine) of 5: \( \delta \) 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), ChlO: 3.21 (3H, s), and 13C NMR (125 MHz, pyridine) (see Table 2).

**Iwensissaponin A (6):** UV \( \lambda_{\text{max}} \) (MeOH) 206 nm, IR (KBr) \( \nu_{\text{max}} \) 3434 (OH), 1645 (C=C) cm\(^{-1}\). Positive ion LC-ESIMS m/z 1095 [M+Na\(^+\)] (calc. for C\(_{34}\)H\(_{31}\)O\(_{14}\)). \(^1\)H NMR (500 MHz, pyridine) of 6: \( \delta \) H 5.94 (1H, d, J=10.4 Hz, H-1), 5.77 (1H, d, J=15 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
13C NMR (125 MHz, CD3OD) (see Table 2).

Verbascoside (Acteoside) (7): UV λmax (MeOH) 220, 332 nm, IR (KBr) νmax 3392 (OH), 1699 (C=O), 1631 (C=C), 1604, 1525 (aromatic ring) cm⁻¹, Positive ion LC-ESIMS m/z 647 [M+Na]+ (calc. for C29H36O15), 1H NMR (500 MHz, DMSO-d6) of 7: δH 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, bs, H-3'), 3.67 (1H, m, H-αa), 3.68 (1H, m, H-3'), 3.45-3.70 (2H, *), 3.36 (1H, m, H-αa), 3.26 (1H, t, J=8.3 Hz, H-2'), 3.15 (1H, *), 1.00 (3H, d, J=5.8 Hz, H-6''), and 13C NMR (125 MHz, CDCl3) (see Table 3). *

(overlapped)

Table 2. 13C NMR (125 MHz, pyridine-d5/5, CD3OD/6) data of compounds 5 and 6

| C/H atom | 5 δc (ppm) | 6 δc (ppm) | C/H atom | 5 δc (ppm) | 6 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       | Glic 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 Glic 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       | Glic 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       |            |            |            |
| OCH3     | 54.1       | -          |            |            |            |
The 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 the most active compound against *C. albicans* and *C. parapsilosis* with an MIC value of 64 µg/mL, as well as ilwensisaponin A inhibited *C. albicans* and *C. krusei* with the same MIC value as ajugol. These active compounds were found to be much more effective against fungi than the *V. mucronatum* extract (Table 4).

**DISCUSSION**

Compound 1 was isolated as a white amorphous powder with the molecular formula C15H24O9 (LC-ESIMS m/z 371 [M+Na]+). An iridoid enolether system (220 nm) in UV spectrum; hydroxyl group (3410 cm⁻¹) and double-bond (1660 cm⁻¹) absorption bands in IR spectra were observed. Compound 1 was identified as ajugol when comparing ¹H and ¹³C NMR spectra with those of ajugol.

Compound 2 (see Figure 1) was isolated as white amorphous powder with the molecular formula C15H22O9 (LC-ESIMS m/z 369 [M+Na]+). An iridoid enolether system (205 nm) in UV spectrum; hydroxyl group (3275 cm⁻¹) and double-bond (1650 cm⁻¹) absorption bands in IR spectra were observed. Compound 2 was identified as aucubin when comparing ¹H and ¹³C NMR spectra with those of aucubin.20,21

Compound 3 (see Figure 1) was isolated as a white amorphous powder with the molecular formula C30H38O15 (LC-ESIMS m/z 661 [M+Na]+). The 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 ¹³C 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 (Figure 1) was isolated as a white amorphous powder with the molecular formula C15H22O10 (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)

| Bacteria          | Staphylococcus aureus | Enterococcus faecalis | Escherichia coli | Pseudomonas aeruginosa | Candida albicans | Candida krusei | Candida parapsilosis |
|-------------------|-----------------------|-----------------------|-----------------|------------------------|------------------|----------------|---------------------|
|                   | ATCC 29213            | ATCC 29212            | ATCC 25922      | ATCC 27853             | ATCC 90028       | ATCC 6258       | ATCC 22019          |
| *Verbascum mucronatum*-MeOH extract | 256       | 128                   | 256             | 256                    | 256              | 128            | 128                 |
| Ajugol            | 128                   | 256                   | 128             | 128                    | 64               | 128            | 128                 |
| Aucubin           | 256                   | 512                   | 512             | 512                    | 256              | 256            | 256                 |
| Lasianthoside I   | >512                  | >512                  | >512            | >512                   | 256              | 256            | 256                 |
| Catalpol          | 256                   | 512                   | 512             | 512                    | 256              | 256            | 256                 |
| Ilwensisaponin C  | >512                  | >512                  | >512            | >512                   | 512              | 256            | 256                 |
| Ilwensisaponin A  | 256                   | >512                  | >512            | >512                   | 512              | 512            | 512                 |
| Verbascoside      | 256                   | 512                   | 512             | 256                    | 256              | 256            | 256                 |
| Ampicillin        | 1                     | 8                     | 2               | -                      | -                | -              | -                   |
| Fluconazole       | -                     | -                     | -               | -                      | 1                | 64             | 8                   |
in the IR spectra were observed. The $^1$H and $^{13}$C NMR spectra of compound 4 were similar to those of catalpol. Thus, compound 4 was identified as catalpol.

Compounds 5 and 6 (Figure 1) were obtained as amorphous compounds with molecular weights of 1104 (LC-ESIMS: m/z 1127 (M+Na)+), and 1072 (LC-ESIMS: m/z 1095 (M+Na)+), as calculated for C$_{52}$H$_{86}$.O$_{32}$, and C$_{52}$.H$_{86}$.O$_{32}$, respectively.

In their IR spectra, the observed absorbances were consistent with the presence of olefinic double bonds. The $^1$H and $^{13}$C NMR data of compounds 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 compound 5, characteristic resonances for anomeric protons were observed at $\delta_1$. 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 $\delta_1$. 104.2 ($\beta$-D-fucopyranose), 105.1 ($\beta$-D-glucopyranose-inner), 104.0 ($\beta$-D-glucopyranose-terminal) and 102.8 ($\alpha$-L-rhamnopyranose), as well as 2 proton signals at $\delta_1$.1.35 (d, $J$ = 4.8 Hz) and 1.68 (d, $J$ = 5.5 Hz), arising from the methyl groups in the sugar moieties. By means of HMBC correlations, the sequence of the saccharidic chain was determined as $[\alpha$-L-rhamnopyranosyl-(1→4)-$\beta$-D-glucopyranosyl-(1→3)]-[$\beta$-D-glucopyranosyl-(1→2]-$\beta$-D-fucopyranoside.

The $^1$H NMR of compound 5 showed 6 tertiary methyl signals at $\delta_1$. 0.88, 0.95, 0.96, 1.07, 1.08 and 1.30. The proton signal at $\delta_1$. 3.21 (3H) was attributed to methoxy protons, and $\delta_1$. 5.46 (br s) to the olefinic proton of the aglycone. It was determined that the aglycone was an oleane-Δ$^2$ type confirmed by presence of $\delta_1$. 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 COSY, HMBC, and HMBC experiments.

The location of the methoxy group was determined using HMBC correlations between methoxy protons and C-11, whereas a chemical shift of C-11 ($\delta_1$. 76.2) was also evident. From the chemical shift of C-11 ($\delta_1$. 76.2) in compound 5, it can be concluded that the methoxy group had an α-configuration as reported for saikosaponin-b$_{\alpha}$. The H-3 methane 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)-($\beta$-D-glucopyranosyl-(1→3))-$\beta$-D-glucopyranosyl)-(1→2)-$\beta$-D-fucopyranosyl]-11-methoxy-olean-12-ene-3β,23,28-triol (=ilwensisaponin C)$_{25}$.

Compound 6 was distinguished from compound 5 by differences in the aglycone parts in $^1$H and $^{13}$C NMR spectra. The $^1$H NMR of compound 6 showed 6 tertiary methyl signals at $\delta_1$. 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), $\delta_1$. 132.9 and $\delta_1$. 5.53 (*), $\delta_1$. 131.9, respectively. Thus, aglycone was identified as an oleane-Δ$^2$ type and no signals of a methoxy group in $^1$H and $^{13}$C NMR spectra of compound 6 were observed compared with those of compound 5.

Due to presence of an oxo-bridge between C-28 and C-13, a chemical shift of C-28 methylene protons ($\delta_1$. 3.33-3.72) appeared in the higher field in comparison with those of C-23 hydroxylated methylene protons ($\delta_1$. 3.70-4.34). Based on this evidence, the aglycone of compound 6 was determined as 13β, 28-epoxyolean-11-ene-3β,23-diol.

As a result, the structure of compound 6 was determined as 3-O-[(α-L-rhamnosyl-(1→4)-($\beta$-D-glucopyranosyl-(1→3))-$\beta$-D-glucopyranosyl)-(1→2)-$\beta$-D-fucopyranosyl]-13β,28-epoxyolean-11-ene-3β,23-diol (=ilwensisaponin A$_{25}$-mimen-goside A).$^{27}$

Compound 7 (Figure 1) 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 contained diverse iridoid glycosides such as ajugol$^{12}$, aucubin$^{28}$, lasianthoside I$^{22}$ and catalpol$^{23}$; saponins such as ilwensisaponin C$^{9}$ and ilwensisaponin A$^{12}$; and phenylethanoid glycosides such as verbascoside.$^{11}$ Ilwensisaponin A has previously been found to be active against Aspergillus fumigatus$^{29}$; it showed moderate antifungal activity in the current study.

CONCLUSIONS

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

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