Determination of Cytotoxic and Anticandidal Activities of Three *Verbascum* L. Species from Turkey: *V. cheiranthifolium* Boiss. var. *asperulum* (Boiss.) Murb. Monorg., *V. pycnostachyum* Boiss. & Heldr and *V. orgyale* Boiss. & Heldr

Purpose of this study is to determine of cytotoxic and anticandidal activities of *Verbascum cheiranthifolium* Boiss. var. *asperulum* (Boiss.) Murb. Monorg., *V. pycnostachyum* Boiss. & Heldr and *V. orgyale* Boiss. & Heldr belonging to *Verbascum* genus growing in Turkey. The cytotoxic effects of methanolic extract of *Verbascum cheiranthifolium* var. *asperulum*, *V. pycnostachyum* and *V. orgyale* species on the cervical (HeLa) and ovarian cancer (Skov-3) cells were investigated using colorimetric assay. The results indicated that methanolic-extract of *V. pycnostachyum* had a promising toxic effect on both cell lines as compared to the other species. Furthermore, this effect was more significant on Skov-3 cells rather than HeLa cells. Anticandidal effects of the methanolic extracts were evaluated in comparison with standard antifungal agents according to Clinical Laboratory Standards Institute (CLSI) reference methods, for the first time here. *V. pycnostachyum* and *V. orgyale* extracts were demonstrated stronger inhibitory effects than the *V. cheriantifolium* var. *asperulum*. Remarkably, *Candida krusei* was inhibited by *V. pycnostachyum* extract at the concentration of the 62.5 µg/mL.

**Key words:** Scrophulariaceae, *Verbascum*, Cytotoxicity, Anticandidal activities
In Turkey, the species endemic species (4,5) endemic ratio (80%) of the genus is very high with 196 species, 6 imperfectly known or doubtful records (2-5). The additional 130 hybrids, the genus is represented by 246 about 360 species throughout world (1). In Turkey, with Verbascum Turkish flora (6,7). One of the well-known species, some of them are widely used in folkloric medicine due to their antimicrobial and anticarcinogenic properties, in Turkish flora (6,7). One of the well-known Verbascum species is V. thapsus L., which has been used for the treatment of several diseases including asthma, spasmodic cough, migraine and earache. Moreover, V. thapsus, V. fruticulosum V. undulatum and V. georgicum had anti-malarial and antiviral effects that were investigated by both in vitro and in vivo studies (6).

It is reported that leaves and flowers of Verbascum species have expectorant, mucolytic and demulcent properties, and they are used to treat respiratory disorders such as bronchitis, dry coughs, tuberculosis, asthma in Anatolia (8,9). Verbascum species are also used to treat hemorrhoids, rheumatic pain, superficial fungal infections, wounds and diarrhea. Furthermore these species demonstrate several inhibitory activities against the murine lymphocytic leukemia and influenza viruses A2 and B. Macerated oil prepared from the flowers is used for reducing earache, applied externally for eczema and other types of inflammatory skin disorders (10).

Verbascum species have some folkloric usages such as sedative and treatment of dysmenorrhea and rheumatalgia. It was also notified the usage for healing wounds in animal care.

Iridoid and neolignan type glycosides, oleanane type terpenes, flavonoids, polysaccharides, saponins, steroids and alkaloids were major compounds isolated from Verbascum species (11). In several bioactivity studies on Verbascum sp. reported that crude extracts of roots, leaves, flowers and aerial parts have been shown anti-proliferative (12), anti-inflammatory (13), antioxidant (14,15), anti-histaminic, anti-fungal, anti-bacterial, (16), wound healing (17), anti-microbial (18) and anti-cancer effects (19).

In the present study, three species belonging to Verbascum genus, were evaluated for their cytotoxic (on cervical and ovarian cancer cell lines) and anticandidal effects for the first time.
Anticandidal activity

Anticandidal activities of the methanolic extracts were evaluated by partly modified reference method of Clinical and Laboratory Standards Institute (CLSI) M27-A2 (21).

*Candida albicans* ATCC 90028, *C. utilis* NRRL Y-900, *C. glabrata* ATCC 66032, *C. tropicalis* ATCC 750, *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used as pathogenic test microorganisms. Stock cultures stored in 50% glycerol at -85°C, were inoculated in Mueller Hinton Agar (Acumedia) plates and incubated at 37°C for 24 h for checking purity and viability. After incubation, selected colonies were suspended in 0.85% NaCl solution and adjusted to McFarland No: 0.5. Serial dilutions of the extracts were prepared in range of 4000 to 7 µg/mL. After incubation at 37°C for 24h, MIC values was determined by visual reading of wells without growing. Amphotericin B (Sigma) and Ketoconazole (Sigma) were used as standard antifungal agents.

### Figure 1

Treatment of either *V. pycnostachyum*, *V. cheiranthifolium* var. *asperulum* or *V. orgyale* extracts with HeLa cells decreased the cell viability in a dose-dependent manner. Bars indicate mean ± standard deviation. All comparisons were made relative to untreated control cells. The significant differences were indicated as *p* < 0.05 using one-way ANOVA. The graphic was created by using GraphPad Prism 6 software. [*p*<0.1; **p*<0.01; ****p*<0.001].

### Figure 2

The percentage of cell viability after treating Skov-3 cells with either *V. pycnostachyum*, *V. cheiranthifolium* var. *asperulum* or *V. orgyale* methanolic-extract. Bars indicate mean ± standard deviation. All comparisons were made relative to untreated control cells. The significant differences were indicated as *p* < 0.05 using one-way ANOVA. The graphic was created by using GraphPad Prism 6 software. [*p*<0.1; **p*<0.01; ****p*<0.001].
RESULTS AND DISCUSSION

Cytotoxicity results

The effects of *V. pynostachyum*, *V. cheiranthifolium* var. asperulum and *V. orygale* methanol-extracts were assessed on HeLa (Figure 1) and Skov-3 (Figure 2) cells after 24 hours incubation with each extract using the MTT assay. The results obtained here indicated that all Verbascum sp. reduced the cell viability of both HeLa and Skov-3 cells in a dose-dependent manner. Particularly, the cell viability of both cell lines was significantly declined after treatment of *V. pynostachyum* extract as compared to other Verbascum sp. that cytotoxic effect was observed at lower concentration (0.5 mg/mL - 44.62% cell viability) on Skov-3 cells rather than HeLa (0.5 mg/mL - 71.54% cell viability).

*V. orygale* methanolic-extract was shown a similar effect on both cell lines; HeLa (1 mg/mL - 30.96% cell viability) (Figure 1C) and Skov-3 (1 mg/mL - 34.22% cell viability) (Figure 2C). On the other hand, a dramatic decrease in cell viability for HeLa was observed after incubation of 0.93 mg/mL *V. cheiranthifolium* var. asperulum methanol-extract (Figure 1B) as compared to the cell viability rate of Skov-3 cells treated with 2.01 mg/mL extract (Figure 2B).

The studies about the isolation of bioactive compounds have been reported that flavonoids, saponins, phenylpropanoid (12) and the phenylethanoid glycosides (22) were isolated although the type of bioactive compounds varies depending on the various *Verbascum* sp. Specifically, the isolation works on methanolic-extract and structure elucidation studies of *V. pynostachyum* were shown that it contained iridoids-glycosides, aukubin, ajugol, ajugosid, harpagoside, phenylethanoid glycoside and verbascoside (10). It has been reported that verbascoside has a hydrophilic character (19) and saponins (23) to possess anti-cancer and antimicrobial activity.

In this study, particularly *V. pynostachyum* species having a significant cytotoxic effect on Skov-3 cells that might be caused by the compounds such as verbascoside. However, in order to explain the relationship between activity-structure, it is necessary to determine the content of bioactive compounds of *V. pynostachyum* methanol-extract.

Anticandidal activity results

Anticandidal activities of the methanolic extracts of *V. cheiranthifolium* var. asperulum, *V. orygale* and *V. pynostachyum* were evaluated by using CLSI M27-A2 reference method. Tested *Candida* species were moderately inhibited by the extracts between the concentrations of the 62.5-4000 µg/mL (minimal inhibitory concentration). Remarkably, *V. pynostachyum* showed strong effects on *Candida krusei* having a MIC value of 62.5 µg/mL. *V. orygale* and *V. pynostachyum* demonstrated better effects than *Verbascum cheiranthifolium* var. asperulum against all tested *Candida* strains. All extracts were assumed to have the MIC values outside of the tested range against *Candida glabrata* ATCC 66032 (Table 1). In the previous study on *Verbascum* species, extract of the *V. sinuatum* L. showed anticandidal effect at the concentration of 32 µg/mL against *C. albicans* (25). In another study methanolic extract of the *V. georigicum* which have antimicrobial constituents reported as a novel antimicrobial raw material (6). According to a scientific review on bioactivities of *Verbascum* species, methanol and ethanol extracts showed strong inhibitory effects on *Candida albicans* and Gram (+) bacteria strains due to the their saponin content (26).

Today, especially in immunocompromised people, *Candida* infections causes major health problems. There are few available systemic antifungal drugs, additionally the rate of drug resistance is increasing dramatically to available systemic antifungal drugs. The search for new natural antifungal agents against pathogenic *Candida* species is extremely important (24).

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REFERENCES


### Table 1. Anticandidal Activity (µg/mL, MIC)

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<tr>
<th></th>
<th>C. albicans</th>
<th>C. tropicalis</th>
<th>C. parapsilosis</th>
<th>C. utilis</th>
<th>C. glabrata</th>
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<td>250</td>
<td>&gt;4000</td>
<td>2000</td>
<td>&gt;4000</td>
<td>250</td>
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<tr>
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<td>250</td>
<td>125</td>
<td>250</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td><em>V. pynostachyum</em></td>
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<td>250</td>
<td>125</td>
<td>250</td>
<td>&gt;4000</td>
<td>125</td>
</tr>
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<td>Amphotericin B</td>
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<td>0.5</td>
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<td>0.031</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
</tbody>
</table>
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