Introduction

The genus *Taxus* L. (Taxaceae), yew, is widely distributed in Europe, North America, Eastern Asia and Asia Minor. There are eight *Taxus* species and two hybrids worldwide and *Taxus baccata* L. is the single representative in Turkey. *T. baccata*, English yew, is an evergreen and widespread shrub commonly used for ornamental landscaping (Davis and Cullen, 1965; Van Rozendall *et al.*, 1999). However, due to its poisonous properties, only few records document the plant as folk medicine, *i.e.* as an abortifacient, antimalarial, and anti-rheumatic and against bronchitis (Bryan-Brown, 1932; Appendino, 1993) as well as against asthma (Singh, 1995).

The genus *Taxus* L. has interested many researchers since the discovery of the anticancer agent paclitaxel (Taxol), a diterpenoid alkaloid originally isolated from the bark of the Pacific yew, *T. brevifolia* (Wani *et al.*, 1971). The drug is the first natural product described that stabilized microtubules and has been approved by the FDA for the treatment of ovarian, breast and non-small-cell lung carcinomas (Rowinsky, 1997). So far, several hundred different taxoids, lignans, flavonoids, steroids and sugar derivatives have been isolated from different parts of various *Taxus* species (Parmar *et al.*, 1999). Our previous phytochemical investigations on the chloroform-soluble portion of the ethanol extract of the heartwood of *T. bac-

Lignans are known to possess various biological activities such as antibacterial, antifungal, antiviral, antioxidant, anticancer and anti-inflammatory effects (MacRae and Towers, 1984). We previously tested the ethanol extract of *T. baccata* heartwood for antimicrobial activity. The extract showed significant activity against gram negative bacteria and against fungi (Erdemoglu and Sener, 2001). In our previous studies, *in vivo* anti-inflammatory and antinociceptive activity and *in vivo* anti-ulcerogenic potency of the isolated compounds from *T. baccata* were investigated (Kupeli *et al.*, 2003; Gurbuz *et al.*, 2004). All compounds, taxoids and lignans, were shown to possess significant antinociceptive activity against *p*-benzoquinone induced abdominal contractions, while only lignan derivatives significantly inhibited carrageenan-induced hind paw edema in mice (Kupeli *et al.*, 2003). Besides, all compounds were shown to possess significant anti-ulcerogenic activity, and the effect of taxiresinol was found to be the most prominent (Gurbuz *et al.*, 2004).

There are a number of reports on lignan derivatives with cytotoxic activity (Habtemariam, 2003; Chang *et al.*, 2000). The aryltetralin lignan (−)-pseudophyllotoxin occupies an unique position among the lignan natural products since a glucopyranoside derivative was recognized as a potent antitumor factor (Jardine, 1980). The cytotoxicity and
antimicrobial activity of these lignans have not been much evaluated so far. This prompted us to investigate cytotoxic and antimicrobial activities of the isolated lignans \((-\text{taxiresinol})\) (1), \((-3'\text{demethylisolariciresinol}-9'\text{hydroxyisopropylether})\) (2) and \((-3'\text{demethylisolariciresinol})\) (3) from \(T.\ baccata\). As related with the above-presented data, this study is designed to investigate \textit{in vitro} cytotoxicity and antimicrobial activity of three lignans isolated from the chloroform-soluble portion of the ethanol extract of the heartwood of \(T.\ baccata\) growing in Turkey.

**Materials and Methods**

**Plant materials**

\textit{Taxus baccata} L. (Taxaceae) was collected from the vicinity of Camlihemsin, Rize, in June 1995. A voucher specimen (GUE 1560) is kept in the Herbarium of the Faculty of Pharmacy, Gazi University.

**Chemical procedures**

Column chromatography (CC) was performed on silica gel (Kieselgel 60, 0.063–0.200 mm, Art. 7734, Merck) and Kieselgel 60 F254 (0.5 mm thickness, Art. 5554, Merck) was used for preparative TLC. Precoated TLC plates (Kieselgel 60 F254) were employed for chromatographic analysis.

The heartwood of the plant was dried under shade and powdered to a fine grade. The material (3078 g) was extracted with 95\% EtOH at room temperature. The ethanolic extract was evaporated under reduced pressure to give a reddish residue. The residue (308.91 g) was partitioned between CHCl3 and H2O. The CHCl3 phase was concentrated (63.54 g). A portion (49 g) of the CHCl3 extract was chromatographed on silica gel and elution was carried out with increasing polarities of different solvents (hexane \(\rightarrow\) acetone \(\rightarrow\) CHCl3 \(\rightarrow\) CH3OH) to give seven main fractions (I–VII). Each fraction was further purified by CC, preparative TLC or recrystallisation. Detailed isolation procedures of compounds 1–3 were described in our previous studies (Erdemoglu, 1999; Erdemoglu et al., 2003).

**Microorganisms**

All microorganism strains used in bioactivity assays were obtained from the microbiological unit of H. E. J. Research Institute of Chemistry, University of Karachi, Karachi, Pakistan.

**Antibacterial assay**

To test for antibacterial activity, the agar-well diffusion method (Cayaugh, 1963) was used. A bacterial lawn was prepared on a nutrient agar plate by dispensing soft agar containing 100 µl cultures. 6 mm diameter wells were made in each plate using a sterile metallic borer. The concentration of the sample was 1 mg/ml of DMSO. 100 µl samples were added into the wells using sterilized pipettes. The plates were incubated at 37 °C for 24 h. Imipenum (\(\text{N-formimidoylthienamycin monohydrate}\)) was the antibacterial reference substance at 10 µg/disc. The antibacterial activity was evaluated by measuring the diameter of the inhibition zones (mm).

**Antifungal assay**

To test for antifungal activity, the agar tube dilution method, a modification of the agar dilution method of Washington and Sutter (1980), was followed. A 1% Saboraud dextrose agar (SDA) was used in the antifungal assay. Test tubes having sterile SDA were inoculated with test samples (200 µg/ml) and kept in a slanting position at room temperature. The fungal culture was inoculated on the slant and growth inhibitions were observed after incubation at 27 °C for 7 d. Control agar tubes were made in parallel and treated similarly except for the presence of test samples. Amphotericin B (10 µg/ml) was the antifungal reference substance against \(\text{Aspergillus flavus}\). Miconazole (10 µg/ml) was used as the reference compound for the other pathogen fungi. The antifungal activity was evaluated by measuring the inhibition of growth (mm) in percent.

**Cytotoxicity assay**

The bioassay for cytotoxicity was performed by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylte-trazolium bromide] assay (Twentyman and Luscombe, 1987). Compounds 1–3 were evaluated for \textit{in vitro} cytotoxicity against a 9-cell line panel. The panel is made up of eight human tumor cell lines (breast, colon, ovary, prostate, and lung) and a normal adult bovine aortic endothelial cell line (ABAE). The cells were seeded in microtiter plates and the test compounds were added for 24 h. Cell proliferation was determined by a tetrazolium dye conversion assay after a 72-h drug exposure and IC\(_{50}\) values for each cell line were determined. Etoposide (4'-demethyllepipodophyl-
lotoxin 9-[4,6-O-(R)-ethylidene-β-d-glycopyranoside]) was used as a cytotoxic standard agent along with this assay.

Results and Discussion

Chromatographic separation of the chloroform-soluble portion of the ethanol extract from the heartwood of T. baccata collected from Camlihemsin, Rize, Turkey yielded lignan type compounds (Fig. 1), a well-known furanoid lignan, (−)-taxiresinol (1), and two dibenzylbutane type lignans, (−)-3′-demethylisolariciresinol-9′-hydroxyisopropylether (2) and (−)-3-demethylisolariciresinol (3), which were firstly isolated by us (Erdemoglu, 1999; Erdemoglu et al., 2003).

In vitro cytotoxicity of 1–3 is summarized in Table I. It contains the mean IC50 value for the 9 cell lines, as well as the IC50 value for the most sensitive (min IC50) and the least sensitive (max IC50) cell line. None of the compounds demonstrated much cytotoxic potency, as the average IC50 value for the 9 cells lines was > 60 µM.

As the result of the antibacterial activity assay, only the chloroform-soluble portion of the ethanol extract from heartwood of T. baccata exhibited moderate antibacterial activity against the gram negative bacteria Pseudomonas aeruginosa. Compounds 1–3 did not show antibacterial activity. As shown in Table II, (−)-taxiresinol (1) showed moderate antifungal activity against Trichophyton longifusus, Microsporum canis and Fusarium solani. (−)-3-Demethylisolariciresinol (3) exhibited good activity against T. longifusus. The chloroform-soluble extract of T. baccata displayed moderate antifungal activity against Trichophyton longifusus and Microsporum canis. Compound 2 gave no inhibition against all strains.

The results of the present study clearly demonstrate that compounds 1 and 3 as lignan derivatives isolated from T. baccata possess antifungal activity. The breadth of the biological activities of lignans has been appreciated recently, especially their anticancer potency (MacRae and Towers, 1984; Jardine, 1980). The anti-inflammatory, anti-nociceptive, anti-ulcerogenic and antifungal potency of the lignans obtained from Taxus baccata should be further evaluated to develop potential new compounds to introduce in modern therapy. In addition, further studies should be made to reveal the mode of action of lignans which might be helpful in understanding the possible roles in human physiology.

Acknowledgements

We are grateful to Ms. Elizabeth A. Yamashita, Director of Oncology Worldwide, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ, USA for determining in vitro cytotoxic activity.

Table I. In vitro cytotoxicity of compounds 1–3 against the Oncology Cell Line Panela.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean IC50 [µM]</th>
<th>Min IC50 [µM]</th>
<th>Max IC50 [µM]</th>
<th>Max/Min IC50 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt; 114</td>
<td>99</td>
<td>&gt; 129</td>
<td>&gt; 1.2</td>
</tr>
<tr>
<td>2</td>
<td>&gt; 59</td>
<td>23</td>
<td>&gt; 112</td>
<td>&gt; 5</td>
</tr>
<tr>
<td>3</td>
<td>&gt; 107</td>
<td>79</td>
<td>&gt; 128</td>
<td>&gt; 1.6</td>
</tr>
<tr>
<td>Etoposide (VP-16)</td>
<td>0.623</td>
<td>0.133</td>
<td>9.1</td>
<td>68</td>
</tr>
</tbody>
</table>

a Results are expressed as IC50 values [µM].

Fig. 1. Structures of investigated compounds; (−)-taxiresinol (1), (−)-3′-demethylisolariciresinol-9′-hydroxyisopropylether (2), (−)-3-demethylisolariciresinol (3).
Table II. Antifungal activity of tested samples.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>1 Growth [mm]</th>
<th>2 Inhibition (%)</th>
<th>3 Growth [mm]</th>
<th>CHCl₃ extract</th>
<th>Standard drugs</th>
<th>Inhibition of standard drugs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichophyton longijfusus</em></td>
<td>50</td>
<td>50</td>
<td>65</td>
<td>30</td>
<td>50</td>
<td>Miconazole</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>Miconazole</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>80</td>
<td>20</td>
<td>80</td>
<td>100</td>
<td>100</td>
<td>Amphotericin B</td>
</tr>
<tr>
<td><em>Microsporum canis</em></td>
<td>40</td>
<td>60</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>Miconazole</td>
</tr>
<tr>
<td><em>Fusarum solani</em></td>
<td>40</td>
<td>60</td>
<td>100</td>
<td>60</td>
<td>100</td>
<td>Miconazole</td>
</tr>
<tr>
<td><em>Candida glaberata</em></td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>Miconazole</td>
</tr>
</tbody>
</table>

a Concentration of sample was 200 µg/ml of DMSO.