Cytotoxicity and antimicrobial activity of *Teucrium scordium* L. (Lamiaceae) extracts

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Cytotoxicity and antimicrobial activity of cyclohexane, dichlormethane and methanol extracts of *Teucrium scordium* subspec. *scordioides* was studied. Cyclohexane and dichlormethane extracts of *T. scordium* possessed high cytotoxicity against MDA-MB-361 cells (IC₅₀ = 130.33 ± 0.1 µg/ml and IC₅₀ = 189.89 ± 3.99 µg/ml, respectively). Dichlormethane extract was more effective against MDA-MB-453 cell line (IC₅₀ = 130.33 ± 0.1 µg/ml). The methanol extract of *T. scordium* possessed no cytotoxicity against breast cancer cell lines, MDA-MB-361 and MDA-MB-453. Herb extracts of *T. scordium* have shown weak antibacterial activity on *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Bacillus subtilis* with no activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Enterococcus faecalis*, and *Candida albicans*.

**Key words:** *Teucrium scordium* subsp. *scordioides*, cytotoxicity, antimicrobial activity.

**INTRODUCTION**

The genus *Teucrium* L. (Lamiaceae) includes about 200 species and subspecies of herbs and shrubs, often aromatic, with a centre of distribution in the Mediterranean basin (Greuter et al., 1986). In the area of central and west Balkan, nine species of this genus was registered (Tutin and Wood, 1972). The herbs of *Teucrium chamaedrys* L. and *Teucrium montanum* L. are the most popular traditional remedies in Balkans used as cholagogue, tonic and antianemic, as well for treatment of diarrhea, leucorrhea, wounds and hemorrhoids. The infusion of aerial parts of *T. scordium* was used as bitter aromatic, cholagogue with wound healing and fever reducing properties (Redžić, 2007; Jarić, 2007).

The experimental data about chemistry and pharmacology of *Teucrium scordium* L. subspp. *scordioides* are scarce. The previous studies have shown the presence of flavonoids (Harborne et al., 1986), and furanoïd diterpenes in the aerial parts (Papanov et al., 1981; Papanov and Malakov, 1982; Papanov and Malakov, 1985). Kovacevic et al. (2001) reported the composition of the essential oil of *T. scordium* with α- and β-pinene as major components. Morteza-Semnani et al. (2007) reported that, the major constitutents of the *T. scordium* essential oil from Iran were beta-caryophyllene, (E)-beta-farnesene and caryophyllene oxide, while Sharififar et al. (2010) reported pulegone and β-caryophyllene. Diterpenes are the best studied; they had very good antifeedant properties, but concerned responsible for hepatotoxicity of *Teucrium* sp. (Kouzi et al., 1994).

The major researches were done in studying the hepatotoxicity of different *Teucrium* sp. because it was noticed that the products containing germander (*T. chamaedrys*) marketed in France for weight control,
caused 30 cases of hepatotoxicity, and one fatality because of excessive hepatic necrosis (Frazier and Krueger, 2009; Kouzi et al., 1994). Three cases of acute hepatitis were reported after using *Teucrium polium* for treating hyperlipidemia and hypoglycaemia (Savvidou et al., 2007; Starakis et al., 2006). *T. chamaedrys* is known because of its hepatotoxicity (Larrey et al., 1992), as well *Teucrium capitatum* (Dourakis et al., 2002) and *Teucrium viscidum* (Poon et al., 2008). Induction of cytchrome P450 and glutathione depletion were the reasons for germander hepatotoxicity, especially because of presence its major furanoneoclerodane diterpene, teucrin A (Kouzi et al., 1994).

Recent studies have shown that *T. polium* extract inhibited cell invasion and motility of human prostate cancer cells through different molecular pathways (Kandouz et al., 2010), and the essential oils from *T. flavum, T. montbretii* sp. *heliotropifolium, T. polium* sp. capitatum and *T. brevifolium* were cytotoxic against CACO-2, C32 and COR-123 human tumour cell lines (Menichini et al., 2009).

Besides very detailed studies of the pharmacological properties of some *Teucrium* sp. (especially *T. chamaedrys* and *T. polium*), there are not any reports about the effects of *T. scordium* L. subsp. *scordioides* Schreb. In this paper, we tested cytotoxicity of *T. scordium* extracts against two breast cancer cell lines, estrogen-dependant MDA-MB-361 and estrogen-non-dependant MDA-MB-453. Also, the antimicrobial activity was tested using agar diffusion test against Gram-positive and Gram-negative bacteria and fungi.

**MATERIALS AND METHODS**

**Plant material**

The aerial parts of *T. scordium* subsp. *scordioides* were collected on locality Oblačina (Prokupje, Serbia) in September 2007, during the period of full flowering, on salty humid meadows ass. *Caricetum distantis*. Professor of Botany Bransilava Lakušić (Faculty of Pharmacy, University of Belgrade) identified the plant species. Voucher specimens (3092 HFF) were deposited in the Herbarium of Institute of Botany, Faculty of Pharmacy, University of Belgrade, Serbia.

**Extraction**

The air-dried, powdered aerial parts (40 g) were extracted with cyclohexane (2 x 400 mL) during three days (two times, successively). After filtration, plant material was dried and extracted with dichloromethane and methanol (2 x 400 mL) using the same procedure. The solvent was evaporated under low pressure and dried to obtain of C₂H₂O (0.39 g), CH₂Cl₂ (0.81 g) and MeOH extracts (2.94 g).

**High performance liquid chromatography (HPLC) analysis**

High performance liquid chromatography (HPLC) separation was performed using an Agilent 1100 series system equipped with a G-1312A binary pump, a G-1328B injector (20 µL loop) and G1315B DAD detector. The column used was a ZORBAX Eclipse XDB-C18 (4.6 × 250 nm, 5 µm) and operated at a temperature of 25°C. A gradient elution was performed with solvent A (H₂O and H₃PO₄, pH=2.8) and B (solvent A: acetonitrile) as follows: 10-25% B (5 min), 25% B isocratic (10 min), 25-30% B (5 min), 30-50% B (5 min), 50-70% B (5 min), 70-100% B (5 min) at a flow rate of 0.8 mL/min. The injection volume was 20 µL. The present compounds were determined on the bases of their retention times and UV spectra, as well by direct comparison with standards when available.

**Cytotoxicity**

The cytotoxicity of cyclohexane, dichloromethane and methanol extracts was tested using MDA-MB-361 (estrogen-dependant) and MDA-MB-453 (estrogen-nondependant) breast cancer cell lines. Stock solutions (50 mg/ml) of extracts made in dimethylsulfoxide (DMSO) were dissolved in corresponding medium to the required working concentrations. Neoplastic MDA-MB-361 cells (7000 cells per well) and neoplastic MDA-MB-453 cells (3000 cells per well) were seeded into 96-well microtiter plates, and 24 h later, after the cell adherence, five different, double diluted, concentrations of investigated extracts or compounds, were added to the wells. Nutrient medium was RPMI 1640 medium, supplemented with L-glutamine (3 mM), streptomycin (100 µg/ml), and penicillin (100 IU/ml), 10% heat inactivated (56°C) fetal bovine serum (FBS) and 25 mM Hepes, and was adjusted to pH 7.2 by bicarbonate solution.

**Determination of cell survival**

Cell survival was determined indirectly by measuring total cellular protein by the Kenacid Blue R (KBR) dye binding method (Clothier, 1995). Briefly, after 72 h of continuous extracts action, medium was discarded and target cells were washed twice with warm (37°C) phosphate buffered saline (PBS). Then target cells were fixed for 20 min with 150 µl of a mixture of methanol and acetic acid (3:1) and stained 2-3 h with 0.04 % Coomassie Brilliant Blue R-250 in 25% ethanol and 12% glacial acetic acid, washed, and bound dye was dissolved in desorbing solution (1M potassium acetate, 70% ethanol). Absorbance (A) at 570 nm was measured 2 h later. To get cell survival (%), the absorbance of the sample with cells grown in the presence of various concentrations of the investigated agent was divided with control optical density (the A of control cells grown only in nutrient medium), and multiplied by 100. It was implied that A of the blank was always subtracted from A of the corresponding sample with target cells. IC₅₀ concentration was defined as the concentration of an agent inhibiting cell survival by 50%, compared with a vehicle-treated control. All experiments were done in triplicates.

**Antimicrobial activity**

Antibacterial and antifungal activities of the cyclohexane, dichloromethane and methanol extracts of *T. scordium* (100 mg/ml) were evaluating by agar diffusion method (Acar and Goldstien, 1996) on selected Gram-positive and Gram-negative bacteria and fungi: *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *M. luteus* (ATCC 10240), *E. faecalis* (ATCC 29212), *B. subtilis* (ATCC 6633BB), *B. cereus* (ATCC11778), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *K. pneumoniae* (NCIMB 9111) and *C. albicans* (ATCC 10259). Each assay in this experiment was repeated twice. Ampicillin (10 µg/tbl), amikacin (10 µg/tbl), and nystatin (100 U/tbl) served as positive controls.
RESULTS AND DISCUSSION

High performance liquid chromatography (HPLC) analysis

The analysis of HPLC chromatograms by comparison of the retention times and UV spectra of selected picks with standard substances have shown the presence of luteolin, apigenin, diosmetin, luteolin-7-O-glucoside, luteolin-7-O-rutinoside and diosmetin-7-O-glycoside in the methanol extract. Harborne et al. (1986) reported previously the free flavone aglycones located externally on the stems and leaves (cirsiliol, cirsilineol, 5-hydroxy-6,7,3′,4′-tetrametoksiflavone), the flavonoid aglycones after the hydrolysis of ethanol extract (apigenin, luteolin and diosmetin), and glycosides of apigenin and luteolin (apigenin- and luteolin-7-O-glucoside, luteolin-7-O-rutinoside and luteolin-7-sambubioside) in the ethanol extract of T. scordium sp. scordioides from Spain. Our results are completely in accordance with previous researches because the distribution of methoxy flavones was dominant in Teucrium sp., while diosmetin was found especially in species of section Scordium. Also, luteolin- and apigenin-7-glucosides were found in almost all tested Teucrium sp., diosmetin-7-rutinoside in some species of section Scordium and Polium. Luteolin-7-rutinoside was very frequent in all Teucrium sp. Vicenin-2 has not been found in the tested methanol extract of T. scordium, which was also the characteristic of section Scordium.

Cytotoxicity

The cytotoxicity of T. scordium on malignant cell lines has not been studied before. Concerning the presence of furanoid diterpenoids and their cytotoxicity (Chang et al., 2006), we tested activity against MDA-MB-361 (estrogen-dependent) and MDA-MB-453 (estrogen-nondependant) breast cancer cell lines. Kandouz et al. (2010) have tested activity against MDA-MB-361 (estrogen-dependant) and MDA-MB-453 (estrogen-nondependant) breast cancer cell lines. We tested activity against MDA-MB-361 (estrogen-dependant) and MDA-MB-453 (estrogen-nondependant).

The results were presented in Table 1. Cyclohexane and dichloromethane extract possessed high activity on estrogen-dependant breast cancer cell lines with the IC$_{50}$=130.33±0.1 μg/ml and IC$_{50}$=189.89±3.99 μg/ml, respectively. Dichloromethane extract was more effective against estrogen-nondependant cells (IC$_{50}$=130.33 ± 0.1 μg/ml). High sensitivity of both estrogen receptor positive (ER+) and negative (ER-) cell lines to cyclohexane and dichloromethane extracts suggesting the presence of the compounds which antiproliferative action is ER dependant. The methanol extract of T. scordium was not cytotoxic against tested cell lines. Because of very few studies concerning antitumour activity of Teucrium species, and significant cytotoxicity in our research, the obtained results are promising and need further research of chemical composition of cyclohexane and dichloromethane extracts.

**Table 1. Cytotoxicity of T. scordium extracts against MDA-MB-361 and MDA-MB-453 (IC$_{50}$ μg/ml ± S. D.).**

<table>
<thead>
<tr>
<th>Extract</th>
<th>MDA-MB-361</th>
<th>MDA-MB-453</th>
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<tbody>
<tr>
<td>Cyclohexane</td>
<td>130.33 ± 0.1</td>
<td>367.28 ± 11.82</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>189.89 ± 3.99</td>
<td>131.01 ± 0.1</td>
</tr>
<tr>
<td>Methanol</td>
<td>&gt;500</td>
<td>371.55 ± 5.28</td>
</tr>
<tr>
<td>Cis-DDR</td>
<td>17.35 ± 0.27</td>
<td>3.96 ± 0.45</td>
</tr>
</tbody>
</table>

Antimicrobial activity

Herb extracts of T. scordium have shown weak antibacterial activity on P. aeruginosa, K. pneumoniae, E. coli and B. subtilis with no activity against S. aureus, S. epidermidis, M. luteus, E. faecalis, and C. albicans. The results are presented in Table 2. All extracts were active against Gram-negative bacteria P. aeruginosa with the zone of inhibition from 6 mm (dichloromethane extract) to 11 mm (cyclohexane extract). This was in accordance with previously published results concerning moderate antibacterial activity and the absence of antifungal activity of T. montanum extracts (Djilas et al., 2006) and isolated essential oils of T. chamaedrys subsp. chamaedrys, T. orientale var. puberulens, and T. chamaedrys subsp. lydium (Küçük et al., 2006). On the contrary, the essential oil and the methanolic extract of T. sauvegi and T. leucocladum possessed significant antifungal properties (Salah et al., 2006; El-Shazly and Hussein, 2004).

Conclusion

According to the results, the most important findings were the cytotoxicity of cyclohexane and dichloromethane extracts of T. scordium. Those extracts exhibited high cytotoxicity against both estrogen receptor positive (ER+) and negative (ER-) cell lines, MDA-MB-361 and MDA-
Table 2. Antimicrobial activity of T. scordium extracts.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Inhibition zones (mm)</th>
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<tbody>
<tr>
<td></td>
<td>Cyclohexane extract</td>
</tr>
<tr>
<td>S. aureus (ATCC 25923)</td>
<td>-</td>
</tr>
<tr>
<td>S. epidermidis (ATCC 12228)</td>
<td>-</td>
</tr>
<tr>
<td>M. luteus (ATCC 10240)</td>
<td>-</td>
</tr>
<tr>
<td>E. faecalis (ATCC 29212)</td>
<td>-</td>
</tr>
<tr>
<td>B. subtilis (ATCC 10259)</td>
<td>-</td>
</tr>
<tr>
<td>B. subtilis (ATCC 6633BB)</td>
<td>7±0</td>
</tr>
<tr>
<td>E. coli (ATCC 25922)</td>
<td>7±0</td>
</tr>
<tr>
<td>P. aeruginosa (ATCC 27853)</td>
<td>11.0±0</td>
</tr>
<tr>
<td>K. pneumoniae (NCIMB 9111)</td>
<td>6.5±0.1</td>
</tr>
<tr>
<td>C. albicans (ATCC 10255)</td>
<td>-</td>
</tr>
</tbody>
</table>

MB-453. The methanol extract of T. scordium was not cytotoxic against tested malignant cell lines. HPLC analysis has shown the presence of flavonoid aglycones luteolin, apigenin, diosmetin, and their glycosides in the methanol extract. Concerning the high and specific cytotoxic activity, the chemistry of cyclohexane and dichlormethane extracts should be studied in detail. This research should be continued and anticancer potential of plant T. scordium studied further.

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REFERENCES


