

Full Length Research Paper

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* subsp. *scordioides* was studied. Cyclohexane and dichlormethane extracts of *T. scordium* possessed high cytotoxicity against MDA-MB-361 cells ($IC_{50}=130.33\pm 0.1 \mu\text{g/ml}$ and $IC_{50}=189.89\pm 3.99 \mu\text{g/ml}$, respectively). Dichlormethane extract was more effective against MDA-MB-453 cell line ($IC_{50}=130.33 \pm 0.1 \mu\text{g/ml}$). 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*, *S. 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 *T. chamaedrys* L. and *T. 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 *T. scordium* L. subsp. *scordioides* are scarce. The previous studies have shown the presence of flavonoids (Harborne et al., 1986), and furanoid diterpenes in the aerial parts (Papanov et al., 1981; Papanov and Malakov, 1982, 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 constituents 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* species (Kouzi et al., 1994).

The major researches were done in studying hepatotoxicity of different *Teucrium* species because it was noticed that the products containing germander (*T. chamaedrys*) marketed in France for weight control,

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Abbreviations: DMSO, Dimethylsulfoxide; FBS, fetal bovine serum; KBR, Kenacid Blue R; PBS, phosphate buffered saline; ER+, estrogen receptor positive; ER-, estrogen receptor negative.

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 *T. 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 *T. capitatum* (Dourakis et al., 2002) and *T. viscidum* (Poon et al., 2008). Induction of cytochrome P450 and glutathione depletion were the reasons for germander hepatotoxicity, especially because of presence its major furanoneoclerodane diterpene, teucriin 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* ssp. *heliotropiifolium*, *T. polium* ssp. *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* species (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-nondependant 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 (Prokuplje, Serbia) in September 2007, during the period of full flowering, on salty humid meadows ass. *Caricetum distantis*. 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 C₆H₁₂ (0.39 g), CH₂Cl₂ (0.81 g) and MeOH extracts (2.94 g).

HPLC analysis

HPLC separation was performed using a 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 x 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), 70-10% B (5 min) at a flow rate of 0.8 mL/min. The injection volume was 20 µL. The present compounds were determinate 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 positive and gram-negative bacteria and fungi: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Micrococcus luteus* (ATCC 10240), *Enterococcus faecalis* (ATCC 29212), *Bacillus subtilis* (ATCC 6633BB), *Bacillus cereus* (ATCC11778), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (NCIMB 9111) and *Candida 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.

Table 1. Cytotoxicity of *Teucrium scordium* extracts against MDA-MB-361 and MDA-MB-453 (IC₅₀ µg/ml ± S. D.).

Extract	Cytotoxicity	
	MDA-MB-361	MDA-MB-453
Cyclohexane extract	130.33 ± 0.1	367.28 ± 11.82
Dichlormethane extract	189.89 ± 3.99	131.01 ± 0.1
Methanol extract	>500	371.55 ± 5.28
Cis-DDR	17.35 ± 0.27	3.96 ± 0.45

RESULTS AND DISCUSSION

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 (cirsiol, 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* ssp. *scordioides* from Spain. Our results are completely in accordance with previous researches because the distribution of methoxy flavones was dominant in *Teucrium* species, while diosmetin was found especially in species of section *Scordium*. Also, luteolin- and apigenin-7-glucosides were found in almost all tested *Teucrium* species, diosmetin-7-rutinoside in some species of section *Scordium* and *Polium*. Luteolin-7-rutinoside was very frequent in all *Teucrium* species. 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-dependant) and MDA-MB-453 (estrogen-nondependant) breast cancer cell lines. Kandouz et al. (2010) have shown the inhibition of cell invasion and motility of human prostate cancer cells through different molecular pathways by *T. polium* extract recently. The essential oils from four *Teucrium* species were cytotoxic against colon adenocarcinoma CACO-2, amelanotic C32 and large lung carcinoma COR-123 (Menichini et al., 2009).

The results were presented in Table 1. Cyclohexane

and dichlormetane extract possessed high activity on estrogen-dependant breast cancer cell lines with the IC₅₀=130.33±0.1 µg/ml and IC₅₀=189.89±3.99 µg/ml, respectively. Dichlormethane extract was more effective against estrogen-nondependant cells (IC₅₀=130.33 ± 0.1 µg/ml). High sensitivity of both estrogen receptor positive (ER+) and estrogen receptor negative (ER-) cell lines to cyclohexane and dichlormethane 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 dichlormethane extracts.

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 (dichlormethane 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. sauvagei* 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 dichlormethane extracts of *T. scordium*. Those extracts exhibited high cytotoxicity against both ER+ and ER- cell lines, MDA-MB-361 and MDA-MB-453. The methanol extract of *T.*

Table 2. Antimicrobial activity of *Teucrium scordium* extracts.

Microorganism	Inhibition zones (mm)					
	Cyclohexane extract	Dichlormethane extract	Methanol extract	Ampicillin (10 µg/disc)	Amikacin (10 µg/disc)	Nystatin (100 U/disc)
<i>Staphylococcus aureus</i> (ATCC 25923)	-	-	-	35.0±7.0	26.5±2.1	n.t.
<i>S. epidermidis</i> (ATCC 12228)	-	-	-	12.0±0	n.t.	n.t.
<i>Micrococcus luteus</i> (ATCC 10240)	-	-	-	33.0±0	n.t.	n.t.
<i>Enterococcus faecalis</i> (ATCC 29212)	-	-	-	16.0±0	n.t.	n.t.
<i>Bacillus subtilis</i> (ATCC 6633BB)	7.0±0	11.0±0.5	-	15.0±0	n.t.	n.t.
<i>Escherichia coli</i> (ATCC 25922)	7.0±0	-	-	20.5±0.7	20.0 ±0	n.t.
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	11.0±0	6.0±0	6.5±0.1	10.0±0	27.5±3.5	n.t.
<i>Klebsiella pneumoniae</i> (NCIMB 9111)	6.5±0.1	-	-	17.0±4.2	n.t.	n.t.
<i>Candida albicans</i> (ATCC 10259)	-	-	-	n.t.	n.t.	20.0±0.87

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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