Digest Journal of Nanomaterials and Biostructures Vol. 8, No. 2, April - June 2013, p. 845 - 854

CYTOTOXICITY AND ANTIMICROBIAL ACTIVITY OF *SATUREJA KITAIBELII* WIERZB. EX HEUFF (LAMIACEAE)

T. STANOJKOVIĆ[c], B. KOLUNDŽIJA[c], A. ĆIRIĆ[b] , M. SOKOVIĆ[b], D.NIKOLIĆ[d], T. KUNDAKOVIĆ[a,*]

[a]*Department of Pharmacognosy, University of Belgrade, Faculty of Pharmacy, Vojvode Stepe 450, Belgrade, Serbia*

[b]*Institute for Biological Research "Siniša Stanković", University of Belgrade, Despot Stephane Bulevard 142, 11060 Belgrade, Serbia*

[c]*Institute for Oncology and Radiology of Serbia, Pasterova 11, 11000 Belgrade, Serbia*

[d]*Department of Medicinal Chemistry and Pharmacognosy, University of Illinois College of Pharmacy, Chicago, Illinois, USA*

Methanolic extract of *Satureja kitaibelii* Wierzb. ex Heuff. (Lamiaceae) and its major compond rosmarinic acid possessed strong antimicrobial activity against tested bacteria and fungi, and antitumour activity against malignant cells, but without antiproliferative effect on healthy cell line MRC5. Minimal inhibitory concentration (MIC) of methanolic extract was ranged between 0.0125-1.25 mg/ml, while minimal bactericidal concentration (MBC) was of 0.2-5.0 mg/ml and fungicidal (MFC) was 0.15-2.5 mg/ml. The rosmarinic acid exhibited better antimicrobial activity then methanol extract. The most sensitive species to rosmarinic acid were *Bacillus cereus* and *Candida kruzei*, while *Micrococcus flavus*, *Trichoderma viride* and *Aspergillus niger* were the most resistant. The methanol extract exhibited strong activity against Fem-x human malignant melanoma cells with an IC50 39.66±2.71 μg/ml, and moderate activity against other cancer cell lines (IC50 from 138.06±0.16 μg/ml against estrogen-dependant breast cancer cell lines MDA-MB-361 to 173.15±0.02 against a human epithelial cervical cancer cells HeLa). Rosmarinic acid arrested G2/M phase cell cycle in Fem-x cells, against which both methanol extract and rosmarinic acid possessed the best cytotoxic activity.

(Received March 10, 2013; Accepted May 23, 2013)

Keywords: Satureja kitaibelii; Rosmarinic acid; Cytotoxicity; Antimicrobial activity.

1. Introduction

The genus *Satureja* L. (Lamiaceae) consists of over 30 species distributed in the eastern Mediterranean region. *Satureja kitaibelii* Wierzb. ex Heuff. was considered as one of the infraspecies within a very polymorphic species of *S. montana* by older authors [1]. Šilić (1979) has shown that *S. kitaibelii* Wierzb. ex Heuff., an annual, semi-bushy aromatic plant growing on arid, sunny and limestone habitats of eastern Serbia, has clear morphological and chorological characteristics which separates this species from the related *S. montana* [2]. The study of Slavkovska *et al*. (2001) confirmed clear difference in composition of essential oils between *S. montana* and *S. kitaibelii*, which also suggested the specificity of *S. kitaibelii*. *S. kitaibelli* has the major importance in eastern Serbia, especially in the area of mountain Rtanj and a spa Soko Banja with a local name „rtanj's tea"[3]. *Satureja montana* and related species like *S. hortensis* and *S. subspicata* are a very important species in Serbian traditional medicine, for the treatment of

*Correspoding author: ktatjana@pharmacy.bg.ac.rs

respiratory diseases (bronchitis, caught), urinary complaints and digestive disorders, as well as the culinary spices. Externally, it is used for skin and mucous inflammation [4,5].

The previous studies were based on essential oil analysis and its antimicrobial activity. *p*-Cymene was a dominant compound of *S. kitaibelii* essential oil [6]. Geraniol and β-elemene were identified as major compounds in four oils isolated from *S. kitaibelii* collected in Serbia [7-9], while limonene predominated in the samples from Bulgaria and Serbia [10,11]. Besides the composition of the essential oil, antioxidant and antimicrobial activities of different extracts and essential oil against broad range of microorganisms have been reported [11-13,6]. Only one compound, acacetin glycoside, was identified from the methanol extract of aerial parts of *S. kitaibelii* [14].

Significant antibacterial and antifungal activity, as well as the composition of the essential oil from the aerial parts, suggests that *S. kitaibelii* could be a new source of plant material with antiseptic properties and the potential for the treatment of upper respiratory tract infections which was the subject of our previous study [6].

Our further studies are dealing with cytotoxicity of *S. kitaibellii* methanol extract and its major compound rosmarinic acid against malignant and healthy cell lines, their antimicrobial activities, as well to elucidate a possible mechanism of rosmarinic acid cytotoxicity. For the potential therapeutically use, the study of cytotoxicity against MRC-5 healthy cell lines was very important. The influence of identified rosmarinic acid on a cell cycle of Fem-x cell line was studied for the first time.

2. Experimental Section

2.1.Chemicals and reagents

Rosmarinic acid was purchased from Sigma-Aldrich (St. Louis, MO). All organic solvents were HPLC grade and were purchased from J.T.Baker (Deventer, The Netherlands). Phosphoric acid was purchased from Merck Chemicals, Germany, Darmstadt. DMSO (Dimethyl sulfoxide), RPMI-1640 medium without phenol red, fetal bovine serum (FBS), 2-[4-(2-hydroxyethyl)1-piperazino]ethanosulfonosäure (Hepes), and L-glutamine were products of Sigma Chemical Co., St. Louis, MO, as well MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide. Luria-broth (LB) medium (L2542) and Streptomycin (P7794) were products of Sigma-Aldrich (St. Louis, MO), while Bifonazole (Bicutrin) $C_{22}H_{18}N_2$ was purchased from Srbolek, Belgrade, Srbbia. Tween 80 was product of Sineks laboratory doo, Belgrade, Serbia. Mueller-Hinton agar (MH) was purchased from Torlak, Belgrade, Serbia.

2.2.Plant extract preparation

The aerial parts of *S. kitaibelii* were collected in July 2010 on mountain Rtanj (Serbia). The plant material was air-dried at room temperature. The air-dried, powdered aerial parts (1 kg) were extracted with cyclohexane (2 x 10 L) during three days on room temperature (two times, successively). After filtration, plant material was dried and extracted with dichloromethane and methanol (2 x 10 L) using the same procedure. The solvent was evaporated under low pressure and dried to obtain of C_6H_{12} (6.2 g), CH_2Cl_2 (10 g) and MeOH extracts (47.10 g). The methanol extract was used for further analysis.

2.3.Quantification of rosmarinic acid in the plant extract

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 × 250 nm, 5 µm) and operated at a temperature of 25 °C. A gradient elution was performed with solvent A (H_2O and H_3PO_4, 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 presence of rosmarinic acid was identified on the bases of its retention

time and UV spectra, as well by direct comparison with standard. Quantification of rosmarinic acid was performed under the same condition; detection wavelength was 320 nm. All chromatographic operations were carried out at room temperature. The calibration curve was constructed with five concentrations of standard solutions of rosmarinic acid. Within the concentration range of 0,100–1 mg/mL, the relationship between the peak area of rosmarinic acid was linear with a regression equation $y = 27071x - 457{,}5$. The retention time of standard substance rosmarinic acid was 18,8 min. The linearity of the calibration curve was verified by the correlation coefficient ($r^2 = 0.9998$). Each measurement was performed in triplicates. A concentration of 5 mg/ml of the methanolic extract of *S. kitaibelii* in acetonitrile and water (40:60) was used to calculate the amount of rosmarinic acid.

2.4.Cytotoxicity assay

2.4.1. Cell lines

The human melanoma Fem-x cells, MDA-MB-361 (estrogen-dependant) and MDA-MB-453 (estrogen-nondependant) breast cancer cell lines, HeLa-a human epithelial cervical cancer cells, LS174-a human colon cancer cell line and MRC-5 human embryonic lung fibroblast cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA).All cancer cell lines were maintained in the recommended RPMI-1640 medium supplemented with 10% heat-inactivated (56°C) fetal bovine serum, l- glutamine (3 mM), streptomycin (100 mg/ml), penicillin (100 IU/ml) and 25 mM HEPES and adjusted to pH 7.2 by bicarbonate solution. Cells were grown in a humidified atmosphere of 95% air and 5% CO_2 at 37°C.

2.4.2. Treatment of cell lines

Stock solutions (100 mg/ml) of methanol extract and rosmarinic acid, made in dimethylsulfoxide (DMSO), were dissolved in corresponding medium to the required working concentrations. Target neoplastic cells Fem-x cells (3000 cells per well), HeLa cells (2000 cells per well), MDA-MB-453 cells (3000 cells per well), MDA-MB-361 (7000 cells per well), and normal human fetal lung fibroblast MRC-5 cells (5000 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 compounds, were added to the wells except for the control cells to which a nutrient medium only was added. Final concentrations achieved in treated wells were 200, 100, 50, 25 and 12.5 μg/ml. The final concentration of DMSO solvent never exceeded 0.5%, which was non-toxic to the cells.

Nutrient medium was RPMI 1640 medium, supplemented with l-glutamine (3 mM), streptomycin (100 mg/ml), and penicillin (100 IU/ml), 10% heat inactivated (56°C) FBS and 25 mM Hepes and was adjusted to pH 7.2 by bicarbonate solution. The cultures were incubated for 72 h.

2.4.3. Determination of cell survival

The effects of extracts on cancer cell survival were determined by MTT test, according to Mosmann (1983) with modification by Ohno and Abe (1991), 72 h upon addition of the compounds, as it was described earlier [15,16]. Briefly, 20 μl of MTT solution (5 mg/ml PBS) were added to each well. Samples were incubated for further 4 h at 37°C in 5% CO_2 and humidified air atmosphere. Then, 100 μl of 10% SDS were added to extract the insoluble product formazan, resulting from the conversion of the MTT dye by viable cells. The number of viable cells in each well was proportional to the intensity of the absorbance of light, which was then read in an ELISA plate reader at 570 nm. Absorbance (A) at 570 nm was measured 24 h later. To get cell survival (%), A of a sample with cells grown in the presence of various concentrations of extracts 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 triplicate.

2.4.4. Flow cytometry analysis

Cellular DNA content and cell distribution were quantified by flow cytometry using propidium iodide (PI). Cells (3×10^5 cells/well) were seeded in 6-well plates and incubated with or without IC50 or 2IC50 concentration of investigated rosmarinic acid for 24 h. For the assay, purchased standard substance was used. After treatment, the cells were collected by trypsinization, and fixed in ice-cold 70% ethanol at -20ºC overnight. The cells were washed next in PBS and pellets obtained by centrifugation was treated with RNase (100 µg/mL) at 37ºC temperature for 30 min and then incubated with propidium iodide (PI) (40 µg/mL) for at least 30 min. DNA content and cell cycle distribution were analyzed using a Becton Dickinson FAC-Scan flow cytometer. Flow cytometry analysis was performed using a CellQuestR (Becton Dickinson, San Jose, CA, USA), on a minimum of 10,000 cells per sample [17].

2.5. Antimicrobial activity

2.5.1. Microorganisms and culture conditions

For the bioassays we used eight bacteria: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Enterobacter cloacae* (human isolate), and Gram-positive bacteria: *Listeria monocytogenes* (NCTC 7973), *Bacillus cereus* (human isolate), *Micrococcus flavus* (ATCC 10240), *and Staphylococcus aureus* (ATCC 6538). Eight fungi were used for antifungal activity: *Aspergillus flavus* (ATCC 9643), *Aspergillus niger* (ATCC 6275), *Aspergillus fumigatus* (ATCC 9197), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112), *Trichoderma viride* (IAM 5061), *Candida albicans* (human isolate), *Candida kruzei* (human isolate). All of the organisms tested were from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research „Siniša Stanković“, Belgrade, Serbia. The micromycetes were maintained on malt agar (MA), bacteria on Mueller-Hinton agar (MH) and cultures were stored at 4°C and subcultured once a month [18].

2.5.2. Microdilution method

In order to investigate the antimicrobial activity of the extracts tested, the modified microdilution technique was used [19,20]. Bacterial species were cultured overnight at 37ºC in Luria-broth (LB) medium. The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The fungal and bacterial cell suspension was adjusted with sterile saline to a concentration of approximately 1.0 x 10^5 in a final volume of 100 µl per well. The inocula were stored at +4°C for further use. Dilutions of the inocula were cultured on solid MH for bacteria and solid MA for fungi to verify the absence of contamination and to check the validity of the inoculum.

Minimum inhibitory concentrations (MICs) determination was performed by a serial dilution technique using 96-well microtitre plates. The investigated rosmarinic acid were dissolved in DMSO/H_2O=50:50 (1 mg/ml) and the methanol extract dissolved in DMSO/H_2O=50:50 (10 mg/ml) and added in broth medium with inoculum. The microplates were incubated for 48 h at 37°C for bacteria and or 72 h at 28°C, for fungi. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs.

The minimum bactericidal concentrations (MBCs) and fungicidal concentrations (MFCs) were determined by serial subcultivation of a 2 µl into microtitre plates containing 100 μL of broth per well and further incubation for 48 h at 37°C or 72 h at 28°C, respectively. The lowest concentration with no visible growth was defined as MBC/MFC respectively, indicating = 99.5%

killing of the original inoculum. Streptomycin and commercial fungicide, bifonazole, were used as positive controls (0.1-2 mg/ml).

3. Results and discussion

3.1.Quantification of rosmarinic acid in the plant extract

HPLC-DAD analysis of methanol extract have shown the presence of rosmarinic acid as a major compound by comparing the retention time and UV spectra with the standards rosmarinic acid under the same HPLC conditions. The estimated rosmarinic acid content in *S. kitaibelii* methanol extract was 0.272 mg/g of plant extract. Rosmarinic acid is characteristic of subfamily Nepetoides of Lamiaceae family, and it was determined previously in ethanol extracts of several *Satureja* species [21]. The previuos studies of Ćetković *et al*. (2007) have shown also the presence of phenolcarboxylic acids in *S. kitaibelii* as well the presence of rosmarinic acid [13].

Our results of rosmarinic acid content were lower then known percentage for other Satureja species. The recent papar of Hadian *et al*. (2010, 2011) have shown high variation in concentration ranged from 0.06-0.69% (w/w) in methanolic extracts of *S. hortensis* from Iran, as well in *S. khuzistanica* (0.59-1.81%) determined using HPTLC [22,23]. Very high content of rosmarinic acid was determined in *S. hortensis* ethanol (2.137±0.223 g/100 g) and lower in acetone extract (0.249 ± 0.011 g/100 g) using HPLC [24]. Tepe and Sokmen (2007) reported 25.02 ± 1.21 mg/g rosmarinic acid in ethanol extracts of *Satureja hortensis*, while Gabor et al. (1999) reported 0.26% rosmarinic acid in ethanolic extract of *S. montana* using TLC-densitometric method [25,26]. According the data of Kosar *et al*. (2003) the concentration of rosmarinic acid in water extract of *S. cuneifolia* was 5.47 ± 0.22 mg/g determined by HPLC method which was also higher then in our tested extract [27].

3.2. Cytotoxicity assay

The cytotoxicity of extracts was tested against Fem-x human malignant melanoma cells, MDA-MB-361 (estrogen-dependant) and MDA-MB-453 (estrogen-nondependant)-breast cancer cell lines, HeLa-a human epithelial cervical cancer cells, LS174-a human colon cancer cell line and MRC-5-a human fetal lung fibroblasts using MTT (Table 1). The methanol extract exhibited strong activity against Fem-x human malignant melanoma cells with an IC50 39.66±2.71 μg/ml, and moderate activity against other cancer cell lines (IC50 from 138.06±0.16 μg/ml against estrogen-dependant breast cancer cell lines MDA-MB-361 to 173.15±0.02 against a human epithelial cervical cancer cells HeLa).

The antiproliferative activity of standard substance rosmarinic acid was almost double IC50 71.77 ± 1.45 μg/ml then the cytotoxicity of methanolic extract against Fem-x cancer cells, but higher against other cell lines. Both, rosmarinic acid and tested extract showed no cytotoxicity against healthy MRC-5-human fetal lung fibroblasts (IC50 >200 μg/ml). Rosmarinic acid, did not exert any cytotoxic action on normal cells incubated for 72 hrs with 50 (μg/ml) of these compounds. Treatment of cells to high concentrations (200 μg/ml) led to a decrease in cell survival by 20% in normal cells.

The positive control cis-DDP had slightly higher cytotoxic activity than the tested compounds. The negative control DMSO had no inhibitory effect on the tested cell lines.

Table 1. Concentrations of Satureja kitaibelii methanol extract and rosmarinic acid that induced a 50% decrease in Fem-x, MDA-MB 361, MDA-MB-453, HeLa, LS174 and MRC5 cell survival (expressed as IC50 (µg/ml)) the compounds were incubated with cells for 72 h.

Extract/Compound	IC_{50} (µg/ml)					
	Fem-x	MDA-MB-361	MDA-MB-453	HeLa	LS174	MRC5
Methanol extract	39.66± 2.71	138.06±0.16	172.25±1.28	173.15±0.02	163.66±0.62	>200
Rosmarinic acid	71.77± 1.45	73.94±1.18	80.24±0.01	114.71±1.28	95.55±0.94	>200
Cisplatin[a]	0.94 ± 0.25	5.86 ±0.35	1.24±0.11	0.72±0.14	2.61±0.11	14.25±0.12

Note: [a]Used as a positive control.
IC_{50} values were expressed as the mean±SD determined from the results of MTT assay in three independent experiments.

3.3. Effect of rosmarinic acid on cell cycle in melanoma Fem-x cells

In continuation of our work, the effect of rosmarinic acid on cell cycle was evaluated using flow cytometric analysis. Figure 1 shows a representative cell-cycle distribution of Fem-x cells incubated in the absence or presence of rosmarinic acid (IC50 or 2IC50) for 24 hours, the approximate doubling time of this cell line. After 24 hr of IC50 rosmarinic acid treatment, cells in the G2/M population increased from 15.52 % to 20.78 % compared to control whereas cells with 2IC50 rosmarinic acid treatment, the G2/M population increased to 25.46%. The increase of cell population at the G2/M phase was accompanied by a decrease of cell population in the G1 phase of the cell cycle (Figure 1). The effect of rosmarinic acid on Fem-x cells appears to be dose-dependent, the higher the dosage the greater the G2/M population increases. Mechanisms of G2/M phase cell cycle arrest by the rosmarinic acid in these malignant cells remain to be elucidated. However, from the results, we speculate that the rosmarinic acid could induce G2/M phase of cell cycle arrest in a p53-dependent manner associated with increase expression of p21 [28]. Whether, these and other proteins are involved in the induction of G2/M cell cycle arrest and inhibition in cell proliferation still need further studies.

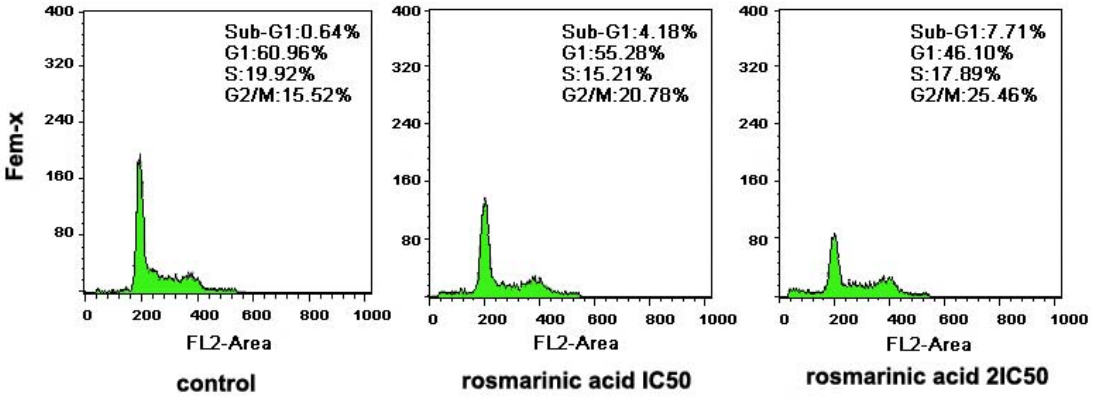

Fig. 1. Cell cycle distribution after 24 h of continuous action of (IC50 or 2IC50) rosmarinic acid in Fem-x cell lines. After a 24 h exposure to compound concentration, cells were harvested and subjected to PI flow cytometry.

Previous study of Ćetojević *et al*. (2004) have show different effects on HeLa, HT-29 and MCF-7 cell lines which depends on polarity of extracts of *S. montana* [29]. Methanol extract of *S.*

montana stimulated proliferation of HT-29 cells, and inhibited proliferation of HeLa cells with no activity against MCF-7 cells. Tested methanol extract of *S. kitaibelii* have shown strong cytotoxic activity against Fem-x cells and moderate activity against other cancer cell lines. The important thing is that both, rosmarinic acid and tested extract showed no cytotoxicity against healthy MRC-5-human fetal lung fibroblasts (IC50 >200 μg/ml). Cytotoxicity of standard substance rosmarinic acid was almost double IC50 71.77 ± 1.45.

Concerning rare studies, rosmarinic acid exhibited very low toxicity with a LD_{50} in mice of 561 mg/kg after i.v. application, and it is rapidly eliminated from human and rat blood circulation after oral administration [30,31]. Results of Lin *et al.* (2012) have shown antiproliferative activity against Hep G2, KB and TSGH 9201 human cancer cells, and COX-2 (induced by 12-O-tetradecanoylphorbol-13-acetate in KB cells) suppressing activity [32].

3.4. Antimicrobial activity

Antimicrobial activity of methanol extract and its major compound rosmarinic acid was tested against selected gram-positive and gram negative bacteria and fungi Table 2. Minimal inhibitory and minimal bactericidal/fungicidal concentration was determined using microdilution method.

Table 2. Minimal inhibitory (MIC mg/ml) and bactericidal/fungicidal concentration (MBC/MFC mg/ml) of compounds tested.

	MIC/MBC (mg/ml)				**MIC/MFC (mg/ml)**		
Bacteria	**MeOH extract**	**Rosmarinic acid**	**Streptomycin**	**Fungi**	**MeOH extract**	**Rosmarinic acid**	**Bifonazole**
Bacillus cereus	0.625/ 2.5	0.0125/ 0.2	0.05/ 0.05/	***Penicillium funiculosum***	0.156/ 2.5	0.0125/ 0.2	0.2/ 0.25
Micrococcus flavus	1.25/ 5.0	0.1/ 0.5	0.05/ 0.1	***P. ochrochloron***	0.156/ 0.313	0.0125/ 0.25	0.15/ 0.2
Staphylococcus aureus	0.625/ 2.5	0.1 0.4	0.05/ 0.1	***Trichoderma viride***	1.25/ 2.5	0.05/ 0.25	0.2/ 0.25
Listeria monocytogenes	1.25/ 5.0	0.025/ 0.4	0.15/ 0.3	***Aspergillus fumigatus***	1.25/ 2.5	0.0125/ 0.25	0.15/ 0.2
Escherichia coli	0.313/ 2.5	0.05/ 0.4	0.1/ 0.2	***A. niger***	1.25/ 2.5	0.025/ 0.25	0.15/ 0.2
Enterobacter cloacae	0.313/ 2.5	0.1/ 0.2	0.1/ 0.3	***A. flavus***	0.625/ 1.25	0.05/ 0.25	0.2/ 0.25
Pseudomonas aeruginosa	1.25/ 2.5	0.1/ 0.4	0.1/ 0.2	***C. albicans***	0.625/ 1.25	0.05/ 0.2	0.1/ 0.15
Salmonella typhimurium	1.25/ 2.5	0.05/ 0.4	0.1/ 0.4	***C.kruzei***	0.313/ 0.625	0.0125/ 0.15	0.05/ 0.15

The methanol extract and its major compound rosmarinic acid showed antibacterial and antifungal activity against all tested species, but on different level. Minimal inhibitory concentration (MIC) was ranged between 0.0125-1.25 mg/ml, while minimal bactericidal concentration (MBC) was of 0.2-5.0 mg/ml and fungicidal (MFC) was 0.15-2.5 mg/ml. The rosmarinic acid exhibited better antimicrobial activity then methanol extract. The most sensitive species were *B. cereus* and *C. kruzei* while *M. flavus*, *T. viride* and *A. niger* were the most resistant. Standard drug used as a positive control, streptomycin was also active against all the

bacteria. Range of MIC for streptomycin is 0.05-0.1 mg/ml and MBC 0.05-0.4 mg/ml. When we compare biological activity of extracts and rosmarinic acid with commercial antibiotic, we could see that tested rosmarinic acid showed lower or similar antibacterial potential, while the methanol extract exhibited slightly higher potential (Table 2). Bifonazole possessed inhibitory activity of 0.05-0.2 mg/ml and fungicidal of 0.15-0.25 mg/ml. The synthetic fungicide exhibited lower or similar antifungal activity as samples tested.

Antimicrobial activity of *Satureja* essential oils and extracts is well-known [33]. Our previous results have show strong antimicrobial activity of essential oil from *S. kitaibelii* with MIC values of 0.10-25 μg/ml [6]. Antimicrobial activities against selected gram-positive and gram-negative bacteria and fungi for methanol extract of *S. kitaibelii* was tested for the first time, and the activity of its major compound rosmarinic acid.

The rosmarinic acid exhibited better antimicrobial activity (MIC 0.0125-0.5 mg/ml) then methanol extract (MIC 0.0125-1.25 mg/ml) which could be compared with standard antibiotics. Ćetković *et al.* (2007) was studied antimicrobial activity of petroleum ether, chlorform, ethylacetat and buthanol extracts of *S. kitaibelii* with MICs and MBCs ranged from 10 to >100 mg/ml [13]. The aqueous extracts of *S. montana* did not show antibacterial activity, and the ethanol extract was not effective against *Salmonella typhimurium* according to Serrano *et al.* [34]. The papers of other authors also suggested strong antibacterial and antifungal activity of *S. montana* extracts [35]. Also, the ethanolic extract of *S. khuzestanica* leaves exhibited antifungal activity against all tested saprophytic fungi (*Aspergillus flavus, A. niger, Penicillium* sp*., Fusarium* sp*., Alternaria* sp*., Rhizopus* sp*.,* and *Mucor* sp*.) Aspergillus flavus, A. niger, Penicillium* sp*., Fusarium* sp*., Alternaria* sp*., Rhizopus* sp*.,* and *Mucor* sp. with MIC values (625-5000 μg/ml) [36].

Tested the three strains of *Bacillus* showed varying degrees of antimicrobial sensitivity when they were treated and incubated with rosmarinic acid for 60 hours. *B. cereus* demonstrated high antimicrobial susceptibility confirming the reported results with rosmarinic acid [37,38]. Also, in our study, *B. cereus* was found to be highly sensitive against rosmarinic acid. Rosmarinic acid was observed to have no antimicrobial activity against *L. monocytogenes* strains tested, LM1, LM2, and LM3, during the 60 hour incubation period [37], it has been in contradiction with our results. Gram-negative pathogens were previously reported to be highly susceptible to rosmarinic acid [39]. Therefore these findings validated that the previous and our studies on effectiveness of rosmarinic acid on *E. coli* are consistent. *Salmonella* species were observed to have high degrees of antimicrobial sensitivity when treated with rosmarinic acid [37].

Report from Gohari *et al.* (2009), revealed the presence of rosmarinic acid isolated from the methanol extract of *H. calycinus* also confirm the antimicrobial activity against *S. aureus*, *E. coli*, *A. niger* and *C. albicans* on range 25-100 ml/ml [39]. The antimicrobial activity of rosmarinic acid has been reported by Salawu *et al.* (2011), also, report from Zhao *et al.* (2011), exhibited good activity on the test microorganisms [40,41].

In conclusion, the results of our experiments with bacteria and fungi indicates that rosmarinic acid has an antimicrobial property, specially against *B. cereus* and *C. kruzei.* Rosmarinic acid is rapidly eliminated from the blood and shows a very low toxicity so, rosmarinic acid could find a position in human therapies using further pharmacological investigations [39].

4. Conclusions

Methanolic extract of *S. kitaibelii* and rosmarinic acid possessed strong antimicrobial activity against tested bacteria and fungi, and antitumour activity against malignant cells, but without antiproliferative effect on healthy cell line MRC5. Rosmarinic acid has shown greater efficacy then methanolic extract *S. kitaibelii*, so the activity of extract could be due to the presence of rosmarinic acid. Rosmarinic acid arrested G2/M phase cell cycle in Fem-x cells, against which both methanol extract and rosmarinic acid possessed the best cytotoxic activity. Further studies of citotoxicity and the mechanisms of anti-tumor activity, as well the chemistry of methanolic extract would be important for better understanding the role of chemical compounds and biological activities of *S. kitaibelii*.

Acknowledgments

Authors wish to express their gratitude to the Ministry of Education and Science of the Republic of Serbia (Projects Nos. 173021, 175011 and 173032) for financial support of this study.

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