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EFFECTS OF *Teucrium* spp. EXTRACTS ON MIGRATORY POTENTIAL AND REDOX STATUS OF HUMAN COLON SW-480 AND BREAST MDA-MB-231 CANCER CELLS

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ABSTRACT. Plant extracts are proved to possess many positive effects in cancer treatment. While many of commonly used therapeutics rely on cytotoxic methodologies, much less are based on positive influence on migrating potential of cancer cells, i.e. on antimetastatic grounds. Here, according to our knowledge, for the first time was investigated influence of *Teucrium montanum* L. and *T. polium* L. methanolic extracts on migratory potential of colorectal cancer cell line SW-480 and human breast carcinoma MDA-MB-231 cell line. The redox status was evaluated on the basis of concentration of superoxide anion radical ($O_2^{\cdot-}$), nitrites and reduced glutathione (GSH), determined spectrophotometrically, while migratory potential of cancer cells was followed by real time cell analysis. Our results showed acute increasing of superoxide anion radical, decreasing of nitrites and decreasing of GSH in SW-480 cells. In MDA-MB-231 cells investigated extracts showed no change in GSH level. Also, methanol extract of *T. montanum* in low concentrations decreased cell migration, while *T. polium* showed antimigratory potential in higher concentrations and in treatment periods longer than 12 hours. Investigated *Teucrium* spp. extracts showed changes in redox status leading to enhancement of oxidative stress. Also, results indicated moderate reducing of migratory potential of tested cancer cells. However, correlation between redox status and antimigratory effects were not observed. According to these findings, the additional investigation of the exact mechanism of antimigratory effect should be performed.

KEYWORDS: breast cancer, colon cancer, migration, redox status, *Teucrium* spp.

INTRODUCTION

Tumors belong to the greatest challenges among the most common diseases. The processes of formation and spread of tumors in distant locations through the human body (metastases) are studied intensively. In many preclinical studies various chemically synthesized substances are used (JEVTIC *et al*., 2014; KALINOWSKA-LIS *et al*., 2008; PETROVIC *et al*., 2015; SABO *et al*., 2004), as well as extracts isolated from plant material (Ravelo *et al*., 2004). Many of these studies are based on toxicity testings, with a focus on *in situ* selectivity towards a certain type of tumor. However, many studies nowadays are engaged in examining of reducing the possibility of tumors to invade the surrounding tissue, i.e. the decrease of

metastatic potential. Among many types of tumors, particularly colon and certain types of breast cancer are aggressive, i.e. able to easily metastasize. One of the first steps in the preclinical studies represents research on cancer cell lines. Therefore, we focused our research on the effects of *Teucrium* spp. methanol extracts in order to investigate the possible oxidative stress and antimigratory effects on colorectal cancer cell line SW-480 and human breast carcinoma MDA-MB-231.

Teucrium spp. plants are commonly used in traditional use since ancient times. Nowadays, *Teucrium* spp. extracts are proved to be useful for treatment of many physiological conditions, e.g. stomach pain, diabetes (MEHRABANI *et al*., 2009; VAHIDI *et al*., 2010), and many others.

It is known that *Teucrium montanum* and *T. polium* contain many secondary metabolites such as phenolic compounds that could be responsible for their cytotoxic and proapoptotic effects (STANKOVIĆ *et al*., 2011). *T. montanum* and *Teucrium polium* showed *in vitro* anticancer activity on HCT-116 (STANKOVIĆ *et al*., 2011) and SW-480 colon cancer cell lines (RAJABALIAN, 2008) as well as on many other cancer cell lines (HAÏDARA *et al*., 2011; RAJABALIAN, 2008; SHAHAT *et al*., 2016; STANKOVIĆ *et al*., 2015). On the other hand, *Teucrium polium* extract showed to possess antimigratory potential on human prostate cancer cells (KANDOUZ *et al*., 2010). According to our knowledge, this is the first work that examines the antioxidative/prooxidative effects and antimigratory potential of *T. montanum* and *T. polium* on colon cancer SW-480 and breast cancer MDA-MB-231.

MATERIALS AND METHODS

Chemicals

Dulbecco`s Modified Eagle Medium (DMEM) and PBS were obtained from GIBCO, Invitrogen, USA. Foetal bovine serum (FBS) and trypsin-EDTA were from PAA (The Cell Culture Company, Pasching, Austria). Dimethyl sulfoxide (DMSO) and nitro blue tetrazolium (NBT) were obtained from SERVA, Heidelberg, Germany. N-1-napthylethylenediamine dihydrochloride was purchased from Fluka chemie GMBH, Buchs, Switzerland. Sulfanilamide and sulphosalicylic acid were purchased from MP Hemija Belgrade, Serbia. 5,5′-dithio-bis(2-nitrobenzoic acid) was purchased from Sigma Chemicals Co., St Louis, MO, USA. All solvents and chemicals were of analytical grade.

Plant material

T. polium was collected in August 2014 on Stara planina Mountain in Eastern Serbia, while *T. montanum* was collected in July 2014 on Goč Mountain in Central Serbia. The collected samples were air-dried in darkness, at room temperature.

Preparation of plant extracts

Prepared plant material (10 g) consisting of aerial parts of *T. polium* and *T. montanum* were transferred to dark-colored flasks. The material was separately soaked in 200 ml of methanol and stored at room temperature. After 24 h, the extracts were filtered through Whatman No. 1 filter paper and the residue was re-extracted with equal volume of solvents. After 48 h, the process was repeated. Combined supernatants were evaporated to dryness under vacuum at 40 °C using Rotary evaporator. The obtained extracts were kept in sterile sample tubes and stored in a refrigerator at 4 °C. For our investigations we used freshly prepared 1 mg/ml stock solutions of extracts in 0.1% DMSO.

Cell Preparation and Culturing

The human colon cancer cell line SW-480 and breast cancer cell line MDA-MB-231 were purchased from the American Tissue Culture Collection (Manassas, VA, USA). The cells were propagated in a humidified atmosphere with 5% CO_2 at 37 °C and maintained in DMEM supplemented with 10% foetal bovine serum, 100 IU/ml penicillin and 100 µg/ml streptomycin. The cells were grown in 75 cm^2 culture bottles until a confluence of 70-80% and after a few passages cells were seeded in assay plates. A number of 10^4 cells per well were seeded in a 96-well plate for determination of superoxide anion radical concentration (NBT assay) and nitrites (Griess assay) and 5 x 10^4 cells per well for determination of reduced glutathione concentration. For migratory potential inhibition assay 2 x 10^4 cells per well were seeded in RTCA xCelligence well.

Redox Status

Determination of Superoxide Anion Radical (NBT Assay)

This method involves estimation of the rate of the reduction of nitrobluetetrazolium (NBT) to nitroblue-formazan in the presence of $O_2^{\bullet-}$ (AUCLAIR and VOISIN, 1985). The cells were seeded in a 96-well plate. After 24 h of incubation, the supplementing medium was replaced with 100 µl of medium containing extracts at final concentration (1, 10, 50, and 100 µg/ml) for 24 and 72 h treatment duration. Untreated cells served as a control. After treatment period, the assay is performed by adding of 10 µl of 5 mg NBT/ml PBS to each well followed with 45 min incubation at 37 °C in 5% CO_2. To quantify the formazan product, formazan was solubilized in 10 µl of DMSO and the resulting color reaction was measured spectrophotometrically at 550 nm on Micro Plate Reader. The amount of reduced NBT was determined by the change in absorbance, based on molar extinction coefficient for monoformazan (15000 M^{-1} cm^{-1}). Results were expressed as nmol/ml.

Nitrite Measurement (Griess Assay)

The Griess colored reaction represents the spectrophotometric determination of nitrites – NO_2^- (indicator of the nitric oxide – NO level) (GRIESS, 1879). The Griess reaction is a process of diazotization in which the NO-derived nitrosating agent (e.g., N_2O_3), generated from the acid-catalyzed formation of nitrous acid from nitrite (or the interaction of NO with oxygen), reacts with sulfanilic acid to produce a diazonium ion that is then coupled to *N*-(1-napthyl)ethylenediamine to form a chromophoric azo product that absorbs strongly at 550 nm. Griess assay is performed at room temperature. All samples were seeded in 96-well microtiter plate, incubated and treated as it was described in NBT assay. After treatment period, 50 µl of 0.1% N-(1-naphthyl)ethylenediamine and 50 µl of 1% sulfanilamide (solution in 5% phosphoric acid) were mixed together (the Griess mixture) immediately prior to application to the plate. The absorbance was measured by using a microplate reader following incubation (usually 5–10 min). The results were expressed in nmol/ml NO_2^- from a standard curve established in each test, constituted of known molar concentrations of nitrites.

Determination of Reduced Glutathione (GSH)

Glutathione assay is based on redox reaction of intracellular GSH with Ellmans reagent, 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), forming yellow product of 5′-thio-2-nitrobenzoic acid (TNB) which strongly absorbs at 405 nm (BAKER, 1990). Similarly to NBT and Griess assays, cells were seeded in 96-well microtiter plate. The cells were treated with

100 µl solutions of tested extracts (0.1, 1, 10 and 100 µg/ml) for treatment of 24 and 72 h. After treatment period the 96-well plate was centrifuged at 1000 g for 10 min, and medium was replaced with 100 µl ice-cold sulphosalicylic acid and kept on ice for 15 min. The next step is centrifugation of the plates at 1000 g for 15 min. 50 µl of supernatant from each well were transferred to another plate with subsequent addition of 100 µl of reaction mixture (1 mM DTNB in 100 mM phosphate buffer, pH 7.4 with 1 mM EDTA), which was prepared closely before the experiment. The rate of colored reaction was quantified spectrophotometrically on microplate reader following incubation of 5 min. The results were expressed in nmol/ml GSH from a standard curve established in each test, constituted of known molar GSH concentrations.

Real Time Cell Analysis

Analysis of cells in real time includes using of xCELLigence system, which represents integration of microelectronics and cell biology. The mechanism of using of this instrument was detailed summarized previously (MONIRI *et al.*, 2015). It is suitable for monitoring of biological processes of adherent cells (viability, proliferation, cytotoxicity, migration). It uses special microtiter plates equipped with gold micro electrodes which in turn measure electrical impedance of the cell population preceding the quantitative information regarding the cell status in real time. Every viable cell that is attached to the gold electrode contributes to the signal of impedance. The more living cells, the higher the impedance.

Determination of Cell Migration

Before experiment it is needed to propagate cells in FBS-free medium for 24 h. Then, 30 µl solution of cells were seeded in 16 well CIM-plate in the upper chamber. In lower chamber it was added 160 µl DMEM supplied with 20% FBS as attractant. *T. montanum* and *T. polium* extracts were added prior to the upper chamber to obtain necessary concentration (1, 10 and 50 µg/ml). As result in this assay we followed CI (cell index) for 24 h, with measuring frequency of 15 minutes.

Statistics

All experimental data were expressed as mean ± standard error (SE). Cell index (CI) for real-time migration estimation (n = 3) was calculated automatically by the RTCA Software Package 1.2 of the RTCA system. Normalizations were performed using the RTCA Software Package 1.2. Biological activity assays are performed in triplicate for each dose. Statistical significance was determined using the one-way ANOVA test for multiple comparisons. A p value < 0.05 was considered as significant. The magnitude of correlation between variables was done using SPSS (Chicago, IL) statistical software package (SPSS for Windows, version 17, 2008).

RESULTS

Redox Status

Concentration of Superoxide Anion Radical ($O_2^{\bullet-}$)

Among all reactive oxygen species superoxide anion radical is one of the most important radical involved in many processes in human cells, because of its content and high reactivity. Superoxide anion radical is an important indicator of ROS level (Hancock, 2001)

and is a key player in oxidative stress processes. In this paper we presented results regarding the level of superoxide on SW-480 and MDA-MB-231 cells (Table 1). Results on SW-480 cells showed that investigated extracts 24 h from treatment increased $O_2^{\bullet-}$ concentration, while after 72 h from treatment extracts induced decreasing of $O_2^{\bullet-}$ level. On MDA-MB-231 cells methanolic extract of *T. montanum* induced increasing of $O_2^{\bullet-}$ 24 h from treatment, while after 72 h concentrations of 1 and 100 µg/ml decreased, and 10 and 50 µg/ml increased $O_2^{\bullet-}$ level. Higher concentration of *T. polium* on MDA-MB-231 after 24 h from treatment showed prooxidative character, i.e. increased $O_2^{\bullet-}$ level, while 72 h from treatment only concentration of 10 µg/ml showed prooxidative character.

Table 1. Effects of methanolic extracts of *Teucrium montanum* and *T. polium* on SW-480 and MDA-MB-231 cell lines, expressed as the $O_2^{\bullet-}$ concentration after 24 h and 72 h of exposure. *$p < 0.05$ as compared to the control cells.

Concentration µg/ml	Superoxide anion radical, $O_2^{\bullet-}$ (nmol/ml)			
	SW-480		MDA-MB-231	
	T. montanum	*T. polium*	*T. montanum*	*T. polium*
	24 h			
0	26.81±0.55	26.81±0.55	27.34±0.27	27.34±0.27
1	25.95±0.88	24.60±0.50	28.61±0.45*	26.92±0.16*
10	28.75±1.00	29.79±2.02	27.29±0.48	26.81±0.22
50	27.44±0.35	32.76±3.20*	28.51±0.59*	37.21±2.17*
100	28.51±0.45*	29.13±3.61	27.51±0.33	30.55±0.62
	72 h			
0	28.35±1.13	28.35±1.13	25.08±0.95	25.08±0.95
1	28.12±1.88	24.44±0.96*	22.44±0.64*	25.07±1.21
10	27.27±3.24	24.77±0.65*	28.19±1.92*	35.75±2.75*
50	27.69±1.22	24.40±0.22*	26.64±0.97	25.92±1.41
100	23.57±0.53*	26.47±1.32	23.83±0.82	26.75±0.70

Concentration of Nitrites (NO_2^-)

Concentration of nitrites indicates quantity of nitrosonium ions (NO^+) in anaerobic conditions. In water, the final product of aerobic phase reaction between nitrogen oxide (NO) and O_2 is nitrogen dioxide (NO_2), which quickly combines with excess of NO, forming nitrogen trioxide (N_2O_3) that is hydrolyzed to nitrites. So, nitrite concentration may indicate level of NO and other reactive nitrogen species in cells (LANCASTER, 2006). Nitrite concentration measurement results are depicted in Table 2. Results obtained on SW-480 cells revealed that *T. montanum* extract significantly decreases nitrites, when compared to control cells 24 and 72 h from treatment. Extract of *T. polium* also significantly reduced nitrite level except in concentration of 100 µg/ml 72 h from treatment. On MDA-MB-231 cells 24 from treatment it was observed that high concentrations of *T. montanum* extract significantly increased nitrites, while after 72 h *T. montanum* extract showed no significant effect on nitrite level. Methanolic extract of *T. polium* in concentration of 50 µg/ml significantly decreased nitrites 24 h from treatment, while after 72 h high concentrations increased nitrite level.

Concentration of Reduced Glutathione (GSH)

Glutathione, GSH (c-Glu-Cys-Gly) is a tripeptide presented in low concentrations in cells with strong antioxidative function (Nordberg and Arner, 2001.). Table 3 represents the effects

of investigated extracts on GSH level in SW-480 and MDA-MB-231 cells. On SW-480 cells *T. montanum* and *T. polium* extracts predominantly decreased GSH concentration. The effect of tested extracts was quite different on MDA-MB-231 cells, when compared to SW-480 cells. *T. montanum* and *T. polium* extracts exerted no effect on GSH changing.

Table 2. Effects of methanolic extracts of *Teucrium montanum* and *T. polium* on SW-480 and MDA-MB-231 cell lines, expressed as the NO_2^- concentration after 24 h and 72 h of exposure. *p < 0.05 as compared to the control cells.

Concentration µg/ml	Nitrites, NO_2^- (nmol/ml)			
	SW-480		MDA-MB-231	
	T. montanum	*T. polium*	*T. montanum*	*T. polium*
	24 h			
0	12.85±0.75	12.85±0.75	14.96±0.29	14.96±0.29
1	8.28±0.20*	6.40±0.40*	14.32±0.46	14.01±0.17
10	7.89±0.29*	8.04±0.37*	14.04±0.31	15.17±0.40
50	8.62±0.32*	10.40±0.67	16.52±0.87*	13.53±0.45*
100	8.13±0.63*	12.81±1.13	16.68±0.74*	15.78±0.36
	72 h			
0	16.81±0.68	16.81±0.68	11.49±0.53	11.49±0.53
1	13.42±3.04	12.66±0.89	10.22±0.20	10.85±0.44
10	11.29±0.94*	14.18±1.84	9.76±0.25	10.33±0.49
50	11.24±0.90*	16.21±2.30	9.69±0.30	12.04±0.53
100	11.55±1.07*	24.23±5.06*	10.47±0.36	18.44±2.75*

Table 3. Effects of methanolic extracts of *Teucrium montanum* and *T. polium* on SW-480 and MDA-MB-231 cell lines, expressed as the GSH concentration after 24 h and 72 h of exposure. *p < 0.05 as compared to the control cells.

Concentration µg/ml	Glutathione, GSH (nmol/ml)			
	SW-480		MDA-MB-231	
	T. montanum	*T. polium*	*T. montanum*	*T. polium*
	24 h			
0	15.87±0.23	15.87±0.23	13.17±0.35	13.17±0.35
1	15.33±0.14	14.57±0.37*	14.31±0.47	11.94±0.51
10	15.29±0.38	14.92±0.21*	12.48±0.46	14.04±0.90
50	15.31±0.46	13.91±0.27*	12.45±0.37	13.69±0.27
100	13.87±0.39*	15.34±0.54	12.89±0.39	13.06±0.42
	72 h			
0	15.21±0.27	15.21±0.27	15.80±0.34	15.80±0.34
1	13.79±0.35*	13.04±0.21*	16.06±0.55	16.04±0.24
10	14.42±0.20	15.19±0.31	15.41±0.45	17.13±1.00
50	13.30±0.22*	13.73±0.29*	15.46±0.35	14.81±0.30
100	14.58±0.47	13.81±0.45*	15.36±0.34	13.93±0.38*

Real Time Cell Analysis and Cell Migration

Effects of *T. montanum* and *T. polium* extracts on migratory potential of SW-480 and MDA-MB-231 cells were monitored and depicted in Figure 1 and Table 4. Presented results

on SW-480 cells revealed that low concentration (1 µg/ml) of *T. montanum* extract reduced, while concentrations 10 and 50 µg/ml enhanced cell migration (Figure 1). On the other hand *T. polium* extract showed significant effect on cell migration reduction in all investigated concentrations. Investigation of *T. montanum* extract on MDA-MB-231 cells revealed that concentrations 10 µg/ml decreased migratory potential, while 1 and 50 µg/ml after 12 h from treatment enhanced cell migration. *T. polium* influenced MDA-MB-231 cell migration in dose and time dependent manner. Concentrations 10 and 50 µg/ml in period from 6 to 12 hours enhanced cell migration, while 18 and 24 h from treatment these concentrations reduced cell migration. On the other hand 1 µg/ml showed promigratory effect during the whole period of treatment.

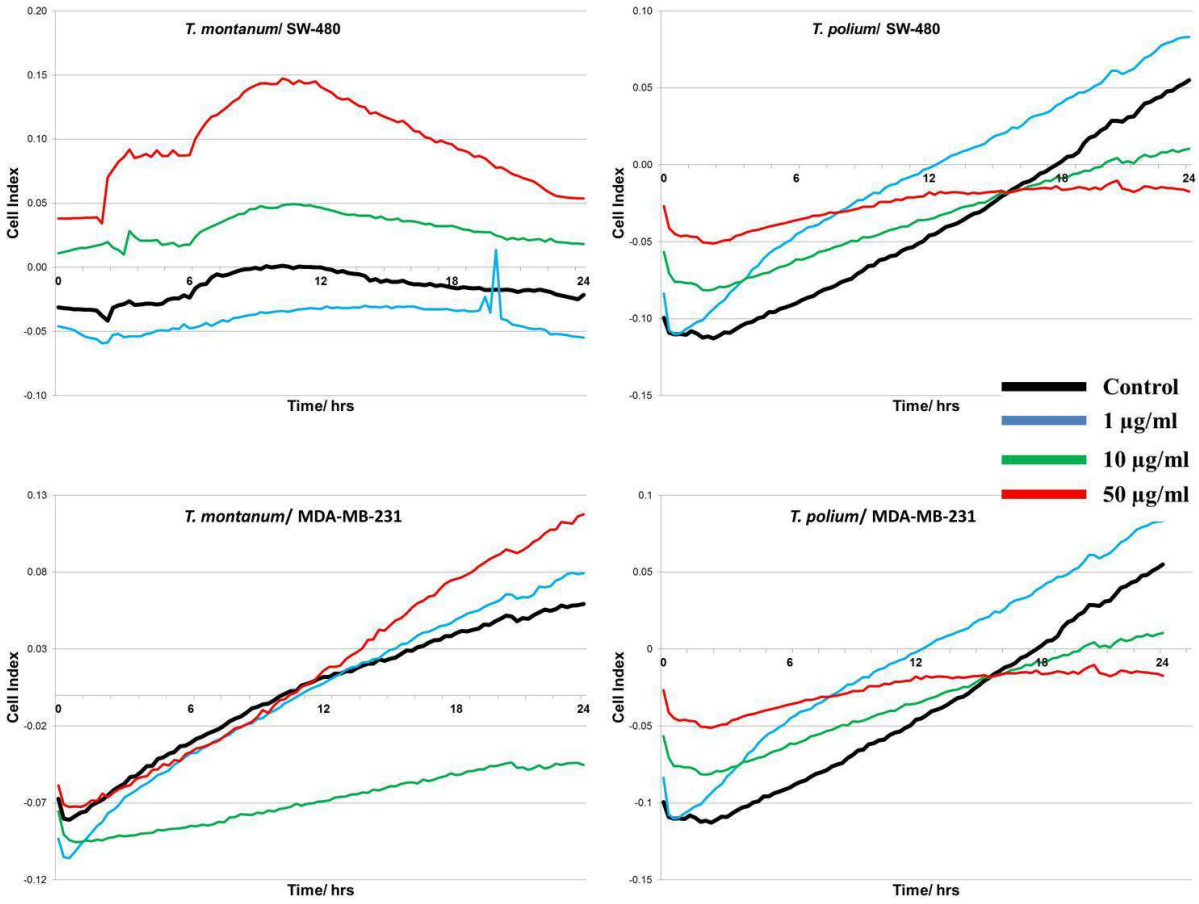

Figure 1. Effects of *Teucrium montanum* and *T. polium* extracts on migration of SW-480 and MDA-MB-231 cell lines.

DISCUSSION

Area of Republic of Serbia is characterized by a very diverse flora with the large number of plants that possess proven medicinal effect (SARIC, 1989.). However, there is not enough investigations on the subject of study of their antitumor effects. Plants of the *Teucrium* genus contain phenolic compounds with strong biological activity (STANKOVIĆ *et al.*, 2011). *Teucrium* genus plants are commonly used in the treatment of conjunctivitis, abscess, gout, respiratory diseases, digestive tract and many others (STANKOVIĆ *et al.*, 2011). In this study we represent results of the effects of methanolic extracts of *T. montanum*, which

is sampled on the serpentine, as well as *T. polium* sampled on the limestone substrate. It was studied influence of extracts on redox status, and migration of SW-480 and MDA-MB-231 cell lines.

Table 4. Effects of *T. montanum* and *T. polium* extracts on migration of SW-480 and MDA-MB-231 cell lines 6 h, 12 h, 18 h and 24 h from treatment.

Concentration µg/ml	Cell index							
	Teucrium montanum				*Teucrium montanum*			
	6 h	12 h	18 h	24 h	6 h	12 h	18 h	24 h
SW-480 cell line migration								
0	-0.02	0.00	-0.02	-0.02	0.02	0.02	0.01	0.00
1	-0.05	-0.03	-0.03	-0.05	-0.01	-0.01	-0.02	-0.02
10	0.02	0.05	0.03	0.02	-0.04	-0.06	-0.08	-0.10
50	0.09	0.14	0.10	0.05	0.00	-0.02	-0.05	-0.07
MDA-MB-231 cell line migration								
0	-0.03	0.01	0.04	0.06	-0.09	-0.05	0.00	0.05
1	-0.04	0.01	0.05	0.08	-0.04	0.00	0.04	0.08
10	-0.08	-0.07	-0.05	-0.04	-0.06	-0.04	-0.01	0.01
50	-0.04	0.02	0.08	0.12	-0.03	-0.02	-0.02	-0.02

T. montanum and *T. polium* exerted some differences in their biological action. The possible cause of the difference in efficiency and biological activity are differences of their phenolic composition. Numerous factors influence the differences in the concentration of phenolic compounds in plants, such as plant maturity, time of sampling, environmental factors, preparation and storage of samples. Environmental factors include the composition of the soil, climate, exposure to the sunlight that has the greatest impact on the composition of flavonoids, as one of the most active group of phenolic compounds in plant material sampled (MACHEIX *et al*., 1990).

Redox regulation has an important role in cell survival, apoptosis and regulation of various signaling pathways in the cell. Changes in ROS/RNS production could modify signal pathways in the cell by direct modification of biomolecules, especially proteins (ENGLAND and COTTER, 2005). Also, level of regulation of the expression of the protein ROS/RNS could change the protein function by regulation of expression of activity of transcription factors, such as NF-kB, AP-1 and HIF-1α (TURPAEV, 2002; ACKER *et al*., 2006; OZBEN, 2007; MATES *et a*l., 2012) or by protein posttranslatory modifications (HENEBERG and DRABER, 2005). The concentration of superoxide anion radical, as one of the most potent reactive oxygen species in cell is an indicator of oxidative stress. The results showed that the extracts of both tested plants after 24 h induced an increase of the concentration of $O_2^{\bullet-}$ in SW-480 cells, while after 72 h $O_2^{\bullet-}$ decreased, which indicates us that extracts possessed acute prooxidative effect. With the extension of time of treatment, the plants showed the protective effect, i.e. antioxidative properties mainly due to prolonged antioxidant activity of protective system in cells and adaptation of the cells to oxidative stress. Results for MDA-MB-231 cells show quiet different effect. *T. montanum* extract induced oxidative stress after 24 h. After 72 h concentrations 1 and 100 µg/ml induced antioxidative effect, while concentrations 10 and 50 µg/ml exerted prooxidative effect. Methanolic extract of *T. polium* in high concentrations after 24 h act as prooxidant, while after 72 h only at a concentration of 10 µg/ml exerts prooxidative effect. The results show that extracts of these herbs act as prooxidants and antioxidants on tested cell lines, which is probably due to the chemical structure and properties of phenols which react at the same time as reducing and oxidizing agents, depending on the environment in which they are located.

The chemical mediator, NO molecule, with very important activity in the body (MONCADA and HIGGS, 1993) is synthesized by nitrite oxide synthase. Treatment of investigated plant extracts lead to a decrease in nitrite concentrations in SW-480 and MDA-MB-231 cells. Results presented in this study confirm the findings of other authors (JEONG and JEONG, 2010; TAE and KIM, 2012) suggesting that some components in extracts (usually a phenolic compounds) are associated with intracellular components and molecules involved in the production of NO, leading to inhibition of this molecule by decreasing in the expression of inducible nitric oxide synthase (iNOS). Extracts with the presence of quercetin, such as the extract of the plant *T. polium*, reduce the production of NO by the cell culture of astrocytes (SOLIMAN and MAZZIO, 1998) or by the inhibition of iNOS protein. Nitric oxide has a short half-life in environments rich in $O_2^{\bullet-}$ which has a great affinity towards NO forming peroxynitrites (WINK and MITCHELL, 1998), which, consequently, can reduce the concentration of NO in the cells treated with the extract of plants. According to STANKOVIĆ *et al*. (2011) *T. montanum* contain higher content of total phenolic compounds than *T. polium*. Considering our results, *T. montanum* mainly induced lesser production of superoxide and nitrites than *T. polium*, which could be related to the content of total phenolic compounds, which mainly act as antioxidants.

Oxidative stress present in cells mainly includes changes in the level GSH system, as the first line of antioxidant defense. Our results show a significant reduction in the concentration of GSH in SW-480 cells under the influence of both plant extracts. MDA-MB-231 cells react a little differently and investigated extracts influenced no change in GSH production.

Migration and invasion of cancer cells represent the initial step in metastasis of cancer, which is the primary cause of death from cancer. The metastasis of primary tumor cells migrates into the surrounding tissue and enter the blood circulation in order to form secondary tumors (BACAC and STAMENKOVIC, 2008). Estimation of the effects of extracts on migratory potential of SW-480 and MDA-MB-231 cells is determined in real time by RTCA xCELLigence system. The results show that methanol extract of *T. montanum* in low concentrations show antimigratory effect (1 μg/ml on SW-480, and 10 μg/ml on MDA-MB-231 cells), while other treatment concentrations act promigratory. On the other hand, *T. polium* extract significantly decreased migratory potential of SW-480 and MDA-MB-231 cells in higher concentrations and in treatment periods longer than 12 hours. Our results do not allow us to correlate redox status with influence on migratory potential of cells. Even though some studies investigated the effect of ROS on cell migration (ALEXANDROVA *et al*., 2006; FINI *et al*., 2008; NISHIKAWA *et al*., 2008), our results indicate that investigated *Teucrium* extracts influence the cell migration through mechanisms possible independent from redox status. KANDOUZ *et al*. (2010) showed that *Teucrium polium* plant extract inhibited cell invasion of human prostate cancer cells via the restoration of the E-cadherin/catenin complex. Such findings refer us to investigate mechanisms alternative to redox status related influence. From the data discussed in this article, and from our measured parameters, *Teucrium* plant extracts showed cytotoxic, proapoptotic and antimigratory potential and they should be considered for serious investigation, especially in elucidating of the effects on antimetastatic potential.

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