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In vitro Antiproliferative activity of *Melissa officinalis* L. (Lamiaceae) leaves essential oil

[Actividad antiproliferativa *in vitro* de aceite esencial de hojas de *Melissa officinalis* L (Lamiaceae)]

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Abstract: In the present study, we investigated the antiproliferative activity of essential oil from leaves of *Melissa officinalis* L. grown in Southern Bosnia and Herzegovina. *In vitro* evaluation of antiproliferative activity of the *M. officinalis* essential oil was carried out on three human tumor cell lines: MCF-7, NCI-H460 and MOLT-4 by MTT assay. *M. officinalis* essential oil was characterized by high percentage of monoterpenes (77,5%), followed by the sesquiterpene fraction (14,5%) and aliphatic compounds (2,2%). The main constituents of the essential oil of *M. officinalis* are citral (47,2%), caryophyllene oxide (10,2%), citronellal (5,4%), geraniol (6,6%), geranyl acetate (4,1%) and β -caryophyllene (3,8%). The essential oil showed significant antiproliferative activity against three cancer cell lines, MOLT-4, MCF-7, and NCI-H460 cells, with GI50 values of <5 , 6 ± 2 and 31 ± 17 $\mu\text{g/mL}$, respectively. The results revealed that *M. officinalis* L. essential oil has a potential as anticancer therapeutic agent.

Keywords: *Melissa officinalis* L.; essential oil; antiproliferative agents; MOLT-4; MCF-7; NCI-H460

Resumen: En el presente estudio, investigamos la actividad antiproliferativa del aceite esencial de las hojas de *Melissa officinalis* L. cultivadas en el sur de Bosnia y Herzegovina. La evaluación *in vitro* de la actividad antiproliferativa del aceite esencial de *M. officinalis* se llevó a cabo en tres líneas celulares de tumores humanos: MCF-7, NCI-H460 y MOLT-4 utilizando el ensayo de MTT. El aceite esencial de *M. officinalis* se caracterizó por un alto porcentaje de monoterpenos (77,5%), seguido de la fracción sesquiterpénica (14,5%) y compuestos alifáticos (2,2%). Los principales constituyentes del aceite esencial de *M. officinalis* fueron citral (47,2%), óxido de cariofileno (10,2%), citronelal (5,4%), geraniol (6,6%), acetato de geraniol (4,1%), y β -cariofileno (3,8%). El aceite esencial mostró una actividad antiproliferativa significativa contra las líneas celulares de cáncer MOLT-4, MCF-7 y NCI-H460, con valores GI50 de <5 , 6 ± 2 y 31 ± 17 $\mu\text{g/mL}$, respectivamente. Los resultados revelaron que el aceite esencial de *M. officinalis* L. tiene potencial como agente terapéutico contra el cáncer.

Palabras clave: *Melissa officinalis* L.; Aceite esencial; Agentes antiproliferativos; MOLT-4; MCF-7; NCI-H460

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INTRODUCTION

Cancer is a complex disease that can affect any part of the body and is the second leading cause of morbidity and mortality worldwide (WHO, 2018). The global cancer burden is estimated to have risen to 18.1 million new cases and 9.6 million deaths in 2018. Worldwide, the total number of people who are alive within 5 years of a cancer diagnosis, called the 5-year prevalence, is estimated to be 43.8 million. Cancer of the lung and female breast are the leading types worldwide in terms of the new cases; for each of these types, approximately 2.1 million diagnoses are estimated in 2018, contributing about 11.6% of the total cancer burden (WHO, 2018). Acute lymphoblastic leukemia is the most common hematological cancer in children and adolescents, representing 20% of all cancers diagnosed in persons aged <20 years in the United States (Ward *et al.*, 2014).

The study of natural products has been the single most successful strategy for the discovery of new medicines used in anticancer therapy and more than two thirds of drugs currently used for cancer treatment have plant origin. Key hallmarks of cancer include resisting cell death, sustained proliferative signaling, and evading growth suppressors (Amin *et al.*, 2009). Therefore, chemotherapy often relies on the characteristics of drugs to reduce the ability of cancer cells to grow and divide and to induce cell damage and death. With the aim of testing possible use as alternative or complementary cancer treatments, several essential oils (EOs) are under investigation for their cytotoxic and antiproliferative actions in cancer cell lines or tumor bearing animals (Edris, 2007; Lesgards *et al.*, 2014). Different mechanisms may account for the reported cytotoxic effects of EOs or their constituents. These include induction of cell death by apoptosis and/or necrosis, cell cycle arrest, and loss of key organelles function.

EOs, secondary metabolites of plants are mixtures of small molecular chemical compounds, mainly terpenes and phenols, with biological action. Essential oils are proved to have various pharmacological effects, such as spasmolytic, carminative, hepatoprotective, antioxidative, antimicrobial, and anticarcinogenic effects (Blowman *et al.*, 2018). EOs have been shown to possess anticancer properties through various mechanisms, including antimutagenic, antiproliferative, antioxidant, and detoxifying capabilities acting on

various pathways in the cancer cell as well as cancer preventative capabilities (Edris, 2007). Specific EO constituents have been shown to enhance the cytotoxic activity of chemotherapy drugs (Docetaxel, Paclitaxel, 5-fluorouracil) in various cell lines, thus increasing the therapeutic window, that is, lowering the required drug concentrations while providing the same effect (Carneseccchi *et al.*, 2004; Legault & Pichette, 2007; Raby & Bishayee, 2009).

Lemon balm, *Melissa officinalis* L. (Lamiaceae) is a well-known used medical plant in Europe. *M. officinalis* L. is a perennial herb and it is indigenous of Southern Europe, Mediterranean region, Western Asia, and North Africa. *M. officinalis* L. is now cultivated worldwide. *M. officinalis* L. occurs naturally in sandy and scrubby areas but has also been reported to grow on damb wasteland, at elevations ranging from sea level to the mountains (Miraj *et al.*, 2017). The leaf of *M. officinalis* L. contains flavonoids (quercitrin, rhamnocitrin, luteolin), polyphenolic compounds (rosmarinic acid, caffeic acid and protocatechuic acid), monoterpenoid aldehyde, monoterpene glycosides, triterpenes (ursolic and oleanolic acids), sesquiterpenes, tannins and essential oil. Thirty-three components were identified representing 89.30% of the total oil in the composition of the leaf. Predominant components in *M. officinalis* L. leaves essential oil are: citronellal, isogeraniol, geraniol acetate, nerol acetate, caryophyllene and caryophyllene oxide (Miraj *et al.*, 2017). Some pharmacological properties have been attributed to *M. officinalis* L. including antimicrobial, antidepressant, hypnotic, hypoglycemic, hepatoprotective and sedative (Miraj *et al.*, 2016). *M. officinalis* L. essential oil has been shown to possess antibacterial, antifungal and spasmolytic activities (Sadraei *et al.*, 2003; Mimica-Dukic *et al.*, 2004), while antitumoral effect has only recently been reported. There has been accumulating evidence supporting the use of *M. officinalis* L. as an anti-cancer agent (de Sousa *et al.*, 2004; Encalada *et al.*, 2011; Saraydin *et al.*, 2012; Unver *et al.*, 2012; Jahanban-Esfahlan *et al.*, 2015; Moaca *et al.*, 2018).

With respect to this topic, this paper focuses on the analysis of the antiproliferative activity of the chemically characterized *M. officinalis* L. essential oil against breast adenocarcinoma (MCF-7) cell line, lung carcinoma (NCI-H 460) cell line and acute lymphoblastic leukemia (MOLT-4) cell line.

MATERIALS AND METHODS

Plant material

The aerial parts of flowering, wild growing, in the first year of growth, plant *Melissa officinalis* L. were collected in June 2015. in the south region of Bosnia and Herzegovina (location: nearby the Jablanicko lake, Latitude N 43°41'30.1"; Longitude E 17°50'12.3"). The taxonomic identification of plant materials, Voucher specimen No.115/15, was confirmed by a senior plant taxonomist, Prof. Samir Đug, and deposited at the Herbarium of the Department of Biology, Faculty of Sciences, University of Sarajevo.

After plant material (3 kg) have been collected, the samples were dried at room temperature and the leaves were manually separated from stems. Leaves were manually crushed and stored under nitrogen atmosphere.

Isolation of the essential oil

A total of 20 g air-dried leaves of *M. officinalis* L. was subjected to hydrodistillation for 2,5 h with 400 mL distilled water using a Clevenger-type apparatus (Klaus Hofmann GmbH, Germany) according to the European Pharmacopoeia (2010). The obtained oil was collected and dried over anhydrous sodium sulphate and stored in a refrigerator at 4-5°C in a dark glass vial prior to analysis. The quantity of the predestilated essential oils was determined volumetrically.

Identification of essential oil components by gas chromatography/mass spectroscopy (GC/MS)

The chemical composition of the essential oils was analyzed using GC/MS system (DSQ II GC/MS with Trace GC Ultra, Thermo Scientific, Palo Alto, USA). Capillary column ZB-5 MS 30 m x 0.25 mm, film thickness 0.2 µm, was used (Phenomenex, Torrance, USA) with temperature program 60°C (1min) rising to 250°C at rate of 4°C/min. Helium was used as carrier gas at a flow rate of 1 mL/min. The temperature of split/splitless injector was 260°C, split ratio was 1:50 and transfer line temperature was set to 270°C for GC/MS analyses. Samples were injected manually (0.1 µL). Mass spectra were recorded at 70 eV (EI), the mass range was 45-350 m/z and Xcalibur version 2.0.7. was used for results processing and quantification. The identity of the components was assigned by obtained GC/MS spectra and retention indices (RI) relative to C₈-C₂₀ n-

alkanes. AMDIS program version 2.62 was used for GC/MS data processing using NIST library version 2.0. Spectra and obtained retention indices were compared with literature (Adams, 2007).

Antiproliferative Activity

The antiproliferative activity of the *M. officinalis* L. essential oil was determined using MTT assay (Sylvester, 2011). *In vitro* evaluation of antiproliferative activity of the *M. officinalis* L. essential oil was conducted in Laboratory for systemic biomedicine, Division of molecular medicine, "Ruder Bosković" Institute in Zagreb. The purpose of this study was to obtain the specific activity profile of lemon balm essential oil by the determination of the activity at different concentrations in an appropriately selected cell panel. For this purpose, well-characterized cell lines with regard to tumor type, genotype, proliferation rates, growth characteristics (solid and hematological) and general drug sensitivity and resistance were used (three cell lines found in the National Cancer Institute cell line screen). A known antitumor compound doxorubicin was tested as a control (referent compound according to NCI protocol), in parallel with the tested compounds (Sylvester, 2011).

Cell culturing

The experiments were carried out on three human tumor cell lines: MCF7 (breast adenocarcinoma, ATCC® HTB-22™), NCI-H460 (lung carcinoma, large cell lung cancer (ATCC® HTB-177™) and MOLT-4 (acute lymphoblastic leukemia ATCC® CRL-1582™). Cell lines were obtained from American Type Culture Collection.

The cells were cultured as monolayers (MCF7 and NCI-H460) or suspension (MOLT-4) and maintained in RPMI 1640 medium, supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100U/ml penicillin and 100 µg/ml streptomycin in a humidified atmosphere with 5% CO₂ at 37°C (Sylvester, 2011).

Proliferation assays

Results were analyzed according to a slightly modified protocol used at the National Cancer Institute (DTP, 2016). The panel cell lines were inoculated in parallel onto a series of standard 96-well micro titer plates on day 0, at 1.2×10⁴ cells/ml (NCI-H460), 3×10⁴ cells/ml (MCF7) or 1×10⁵

cells/ml (MOLT-4), depending on the doubling times of specific cell line. Test substances were then added at 5 µg/ml, 10 µg/ml, 50 µg/ml, 100 µg/ml, 500 µg/ml or 1000 µg/ml concentrations and incubated for a further 72 hours. Doxorubicin was used in parallel as a control, reference compound and was added in five 10-fold dilutions (10^{-10} to 10^{-6} M). Working dilutions were freshly prepared on the day of testing. The solvent dimethyl-sulphoxide (DMSO) was also tested for eventual inhibitory activity by adjusting its concentration to be the same as in working concentration (DMSO concentration never exceeded 0.1%). After 72 hours of incubation, the cell growth rate was evaluated by performing the MTT assay, which detects dehydrogenase activity in viable cells. The MTT Cell Proliferation Assay is a colorimetric assay system, which measures the reduction of a tetrazolium component (MTT) into an insoluble formazan product by the mitochondria of viable cells. For this purpose, the substance treated medium was discarded and MTT was added to each well at a concentration of 20 µg/40 µl. After four hours of incubation, the precipitates were dissolved in 160 µl of DMSO. The absorbance (A) was measured on a microplate reader at 570 nm. The absorbance is directly proportional to the cell viability. The percentage of growth (PG) of the cell lines was calculated according to one or the other of the following two expressions:

$$\begin{aligned} &\text{If } (A_{\text{test}} - A_{\text{tzero}}) \geq 0 \text{ then:} \\ &\text{PG} = 100 \times (A_{\text{test}} - A_{\text{tzero}}) / (A_{\text{cont}} - A_{\text{tzero}}) \\ &\text{If } (A_{\text{test}} - A_{\text{tzero}}) < 0 \text{ then:} \\ &\text{PG} = 100 \times (A_{\text{test}} - A_{\text{tzero}}) / A_{\text{tzero}} \end{aligned}$$

Where:

A_{tzero} = the average absorbance before exposure of cells to the test compound

A_{test} = the average absorbance after the desired period of time (72 h)

A_{cont} = the average absorbance after 72 hours with no exposure of cells to the test compound

Each test point was performed in quadruplicate in minimum three individual experiments. The results are expressed as concentration-response graph. The results are also expressed as GI_{50} , a concentration necessary for

inhibition of 50% of cell growth. Each result is a mean value from three separate experiments. The GI_{50} values for each compound are calculated from dose-response curves using linear regression analysis by fitting the test concentrations that give PG values above and below the respective reference value (e.g. 50 for GI_{50}). Therefore, a "real" value for any of the response parameters was obtained only if at least one of the tested drug concentrations falls above, and likewise at least one falls below the respective reference value. If, however, for a given cell line all of the tested concentrations produce PG's exceeding the respective reference level of effect, then the lowest tested concentration is assigned as the default value. In screening data report, that default value is preceded by a "<" sign, signifying that the "real" value is something "less-than" the lowest tested concentration.

Likewise, if none of the tested concentrations produces the required PG reference level of effect or greater, then a ">" sign precedes the printed default value (which is the highest tested concentration), signifying that the "real" value is something "greater-than" the highest tested concentration (Body & Kenneth, 1995; Sylvester, 2011; DTP, 2016).

RESULTS

Chemical composition analysis

The oil of the leaves of *M. officinalis* L. isolated by hydrodistillation was of yellow-pale color with an agreeable perfumery odor, in total yield of 0.10% (v/w) on dry weight basis.

Qualitative and quantitative analytical results were obtained using GC-MS technique. Table No.1 shows the compounds identified in the oil of *M. officinalis* L. in order of elution on ZB-5 MS capillary column, the percentage content of the individual components, retention indices and chemical class distribution are summarized.

A total of 27 compounds were identified, representing 94.2% of the total content of the *M. officinalis* L. essential oil. This oil was characterized by high percentage of monoterpenes (77.5%), especially oxygenated monoterpenes (57.1%), followed by monoterpene alcohols (14.6%), monoterpene esters (4.8%) and monoterpene hydrocarbons (1.0%). In contrast, the sesquiterpene fraction was lower (14.5%); the oxygenated hydrocarbons (10.2%) were detected in higher concentration than the hydrocarbons (4.3%). Alipha-

tic compounds represented 2.2% of the total oil.

Table No.1
Chemical Composition of *M. officinalis* L. essential oil

Pick no.	Components ^a	RT (minutes)	RI ^b	RI ^c	percentage (%)
1	1-Octen-3-ol	6.52	974	981	0.9
2	6-Methyl-5-hepten-2-on	6.65	988	986	1.3
3	Myrcene	6.79	989	990	0.2
4	p-Cymene	7.82	1020	1028	0.5
5	1,8-Cineole	8.06	1026	1036	0.4
6	trans- β -Ocimene	8.43	1044	1049	0.3
7	Linalool	10.12	1095	1100	2.1
8	α -Thujone	10.38	1101	1109	1.0
9	β -Thujone	10.74	1112	1121	0.3
10	Camphor	11.74	1141	1152	1.7
11	Citronellal	11.84	1148	1155	5.4
12	Isomenthone	12.13	1159	1163	0.9
13	trans-Verbanol	12.76	1178	1181	1.2
14	Terpinen-4-ol	12.83	1180	1183	0.4
15	α -Terpineol	13.31	1186	1196	0.3
16	Nerol	14.26	1222	1226	1.5
17	Citronellol	14.33	1223	1228	1.5
18	Citral	14.76	1235	1241	47.2
19	Carvone	14.95	1239	1247	0.2
20	Geraniol	15.13	1249	1252	6.6
21	Methyl citronellate	15.36	1255	1259	0.7
22	Thymol	16.49	1289	1291	0.9
23	Carvacrol	16.77	1298	1298	0.1
24	Geranyl acetate	19.34	1379	1378	4.1
25	β -Caryophyllene	20.71	1417	1420	3.8
26	Caryophyllene oxide	25.69	1582	1582	10.2
27	α -cadinol	27.8	1650	1656	0.5
total identified		94.2			
monoterpene hydrocarbons					1.0
monoterpene alcohols					14.6
oxygenated monoterpenes					57.1
monoterpene esters					4.8
sesquiterpene hydrocarbons					4.3
oxygenated sesquiterpenes					10.2
aliphatic compounds					2.2

a Compounds listed in order of elution from a ZB-5 MS column. b Literature retention indices.

^c indices relative to C8-C20 n-alkanes on the ZB-5 MS column

As shown in Table No. 1, oxygenated monoterpenes such as citral (47.2%) and citronellal (5.4%), alcohol geraniol (6.6%) and ester geranyl acetat (4.1%) were the major components within the monoterpene fractions. Caryophyllene oxide (10.2%) and β -caryophyllene (3.8%) were dominated by the sesquiterpene fractions.

Antiproliferative evaluation

Results of the antiproliferative evaluation of tested essential oil are presented by Figure No. 1. Essential oil of the *M. officinalis* at 72 hours after treatment

had inhibitory potential on proliferation of tested cancer cells. The results of the antiproliferative activity suggested that *M. officinalis* L. essential oil induced a dose-dependent inhibition of cell viability in all tested tumor cell lines, although each culture showed different sensitivity. This oil demonstrated also a strong cytotoxic effect. A negative percentage indicates cytotoxicity following drug treatment where -100% shows no cells survived the treatment at the specific drug concentration. Results of the antiproliferative evaluation of the doxorubicin, as referent drug, are presented by Figure No. 2.

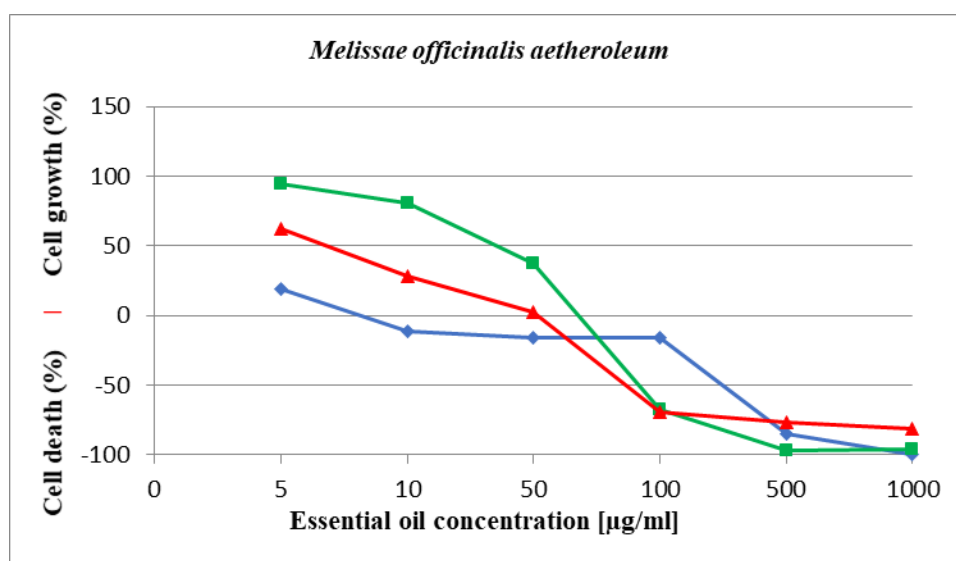


Figure No. 1
Growth inhibition of MOLT-4, NCI-H460 and MCF7 cells *in vitro*, assessed by MTT assay 72 hours after the addition of *M. officinalis* L. essential oil

Table No. 2 presents the GI_{50} values which were determined from the dose-response curves of the oil and the used anticancer drug on MOLT-4, MCF-7 and NCI-H460 cells lines. The strongest antiproliferative activity of *M. officinalis* L. essential oil was observed against MOLT-4 cell line ($GI_{50} < 5$ µg/ml) followed by MCF-7 cell line (GI_{50} 6 µg/ml) and NCI-H460 cells (GI_{50} 31 µg/ml). The strongest

cytotoxic activity of *M. officinalis* L. essential oil was observed against solid tumor cell lines (MCF-7 and NCI-H460). At the applied dose of 100 µg/ml of *M. officinalis* L. essential oil, cytotoxic effect was observed against 70% of MCF-7 and NCI-H460 cell lines, whereby 16% of hematological tumor cell lines (MOLT-4) showed cytotoxicity (Figure No. 1).

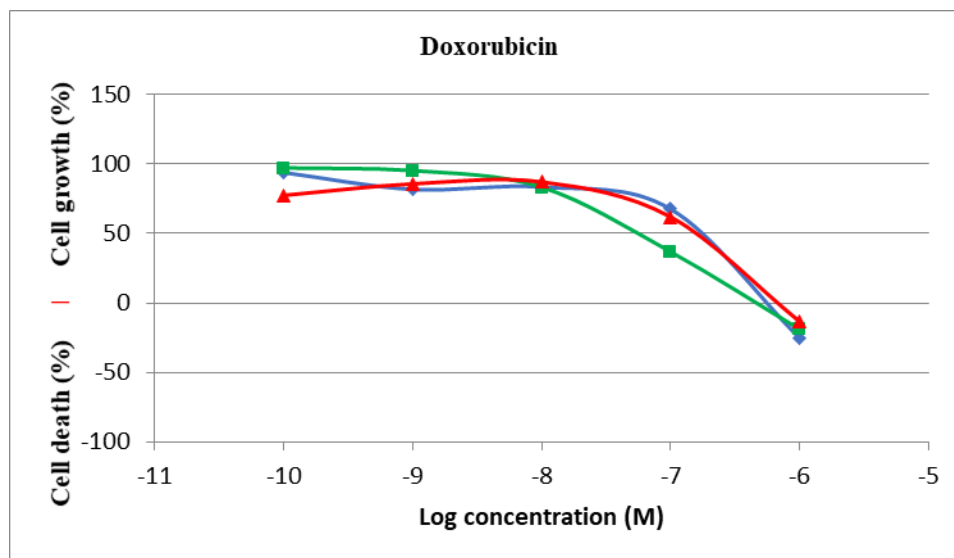


Figure No. 2
Growth inhibition of MOLT-4[■], NCI-H460[■] and MCF7[▲] cells *in vitro*, assessed by MTT assay 72 hours after the addition of doxorubicin

Table No. 2
In vitro antiproliferative activity expressed as GI₅₀^a values (µg/ml) against MOLT-4, MCF-7 and NCI-H460 tumor cell lines.

Compound	GI ₅₀ ^a (µg/ml)		
	MOLT-4	MCF-7	NCI-H460
<i>Melissae aeth.</i>	<5	6±2	31±17
<i>Doxorubicin</i>	0,02±0,01 (0,003±0,002 µM)	0.08±0.03 (0.15±0.04 µM)	0.03±0.01 (0.05±0.01 µM)

^a GI₅₀±SD: the concentration that causes 50% growth inhibition, as assessed by MTT assay, 72 hours after the addition of the test substances. The results are shown as mean±SD from three independent experiments

DISCUSSION

The above results show that chemical composition of the *M. officinalis* L. essential oil, native from Bosnia, was dominated by citral (47.2%), caryophyllene oxide (10.2%), citronellal (5.4%), geraniol (6.6%), geranyl acetate (4.1%) and β-caryophyllene (3.8%).

These results are in accordance with previous published data on lemon balm essential oils in Serbia (Bozin *et al.*, 2006) and Slovakia (Holla *et al.*, 1997). Basta *et al.* (2005) reported that an oxygenated sesquiterpene, caryophyllene oxide (12.6%) was the most abundant constituents in the oil

of *M. officinalis* L. from Greece. Also, caryophyllene oxide was identified at 10.0% in oil from Egypt (Shalaby El-Gengaihi & Khattab, 1995). However, recent studies of lemon balm oil composition from different regions showed that composition of major components may vary, e.g. de Sousa *et al.* (2004) reported higher concentrations of geranial (47.32%) and neral (39.28%), but remarkably lower concentrations of caryophyllene oxide (1.17%), geranyl acetate (1.51%) and β -caryophyllene (0.94%) with absence of geraniol and citronellal in lemon balm essential oil from Brazil (de Sousa *et al.*, 2004).

Our data demonstrated that *M. officinalis* L. essential oil inhibited the viability of MOLT-4, MCF-7 and NCI-H460 cell lines in a concentration-dependent manner. At the concentrations ranging from 5 to 1000 $\mu\text{g/ml}$ a significant effect was observed for each cell line MOLT-4, MCF-7 and NCI-H460 with GI_{50} values $<5 \mu\text{g/ml}$, $6\pm 2 \mu\text{g/ml}$, $31\pm 17 \mu\text{g/ml}$, respectively. The strongest antiproliferative activity of *M. officinalis* L. essential oil was observed against MOLT-4 cell lines ($\text{GI}_{50}<5 \mu\text{g/ml}$), resulted in $>80\%$ of growth inhibition in treated cells at the lowest applied dose (5 $\mu\text{g/ml}$). This account for the high toxicity profile of *M. officinalis* L. essential oil which requires a lower test-concentrations compared to other evaluated cell lines. As proposed by previous studies (Sylvestre *et al.*, 2006) that performed the cytotoxic effect of essential oils, GI_{50} values between 10-50 $\mu\text{g/mL}$ represent a strong antiproliferative activity. Moreover, GI_{50} values between 50-100, 100-200, and 200-300 $\mu\text{g/mL}$ indicate moderate, weak, and very weak antiproliferative properties, respectively. According to these statements, *M. officinalis* L. essential oil has a strong antiproliferative activity. Furthermore, *M. officinalis* L. essential oil in low concentrations (5-10 $\mu\text{g/mL}$) demonstrated antiproliferative activity for all tested cell lines, but in higher concentrations ($>50 \mu\text{g/mL}$ for NCI-H460 and MCF-7 cell lines, and $>10 \mu\text{g/mL}$ for MOLT-4 cell line) exhibit cytotoxic activity. Solid tumor cell lines (MCF-7 and NCI-H 460) were more sensitive to higher concentrations of *M. officinalis* L. essential oil than hematological tumor cell lines (MOLT-4). Although, the strongest antiproliferative activity of *M. officinalis* L. essential oil was observed against MOLT-4 cell lines, it can be noticed that plateau effect of cytotoxicity (16%) is observed in concentrations of 10-100 $\mu\text{g/mL}$. For all tested cell lines, a high level of cytotoxicity (76%-

96%) was observed in concentrations of 500-1000 $\mu\text{g/mL}$ *M. officinalis* L. essential oil.

The study accomplished by de Sousa and coworkers on lung (A549), colon (Caco-2), breast (MCF-2) and leukemia (HL-60 and K562) human cancer cell lines and in a mouse melanoma cell lines (B16F10) corroborates our results, according to which *M. officinalis* L. essential oil also showed antitumoral activity in a concentration-dependent manner. Apoptosis inducing effects of *M. officinalis* L. essential oil in glioblastoma multiforme cells (GBM) has also been reported (de Sousa *et al.*, 2004). A study by Jahanban and coworkers showed the significant results of hydroalcoholic extracts of *M. officinalis* L. (MOE) towards A549 (lung non-small cell cancer lines), MCF-7, PC-3 (prostate adenocarcinoma) and SKOV3 (ovarian cancer cells) (Jahanban-Esfahlan *et al.*, 2015). In all cancer cells, MOE reduces the cell viability to values below 33%, even at the lowest dose applied (5 $\mu\text{g/ml}$). The 50% ethanolic extract reduced colon cancer cell line proliferation (HCT-116) to values close to 40%, even at the lowest dose tested (5 $\mu\text{g/ml}$) (Encalada *et al.*, 2011). Comparing results obtained from our previous study, which tested antiproliferative activity of *Lavandula angustifolia* Mill. essential oil it is noticed that the *M. officinalis* L. essential oil has a significantly more potent effect than *L. angustifolia* essential oil on antiproliferation of tumor cells. *L. angustifolia* essential oil showed antiproliferative activity to MOLT-4, MCF-7 and NCI-H460 cancer cell lines with GI_{50} values that are significantly higher than *M. officinalis* L. essential oil GI_{50} values (Nikšić *et al.*, 2017.).

In-vitro antiproliferative and cytotoxic activity of essential oils had been described already (Setzer *et al.*, 1999; Buhagiar *et al.*, 1999; Angelopoulou *et al.*, 2001; Hayes & Markovic, 2002; Moteki *et al.*, 2002). In some cases, this activity was attributed to specific components of the oil. The chemical complexity of the essential oils as a phytocomplexes contributes to its biological effects, since each constituent takes part in the overall outcome and may modulate the effects of the others. On the other hand, studies on individual ingredients might report results that do not recapitulate the effect of treatment with the phytocomplexes as a whole.

The above results on chemical composition and antiproliferative activity of the oil indicate that these effects may be attributed by the presence of

specific components such as citral (47.2%), citronellal (5.4%) and geraniol (6.6%). The EO component citral has been shown to induce phase II enzymes in a dose-dependent manner (Nakamura *et al.*, 2003). The mechanism of action of citral is due to its geraniol isoform component (Nakamura *et al.*, 2003). Recent studies have shown citral to inhibit cell intracellular levels of oxygen radicals and, consequently, by the modulation of cellular oxidative status, leading to reduction of cancer cell proliferation and ultimately resulting in cell death (Kapur *et al.*, 2016; Sanches *et al.*, 2017). Citral was also shown to induce caspase activation and subsequent apoptosis induction in several cancer cell types, including colorectal cancer and glioblastoma (Dudai *et al.*, 2005; Queiroz *et al.*, 2014; Sheikh *et al.*, 2017). Other studies have shown that citral is a key component of the essential oils extracted from lemon balm with antiproliferative effect in several leukemia cell lines (P388, NB4, MOLT-4) via the induction of apoptosis (Xia *et al.*, 2013). Recent study demonstrated that citral nanostructured lipid carrier inhibited the proliferation of breast cancer cells *in vitro* (MDA MBA-231 human breast cancer cells), majorly through the induction of apoptosis, anti-metastasis, anti-angiogenesis potentials, and reducing the tumor weight and size without altering the therapeutic effects of citral (Nordin *et al.*, 2019).

Geraniol has been shown to inhibit the proliferation of human colon cancer cells *in-vitro* (Carnesecchi *et al.*, 2001) and the growth of pancreatic (Burke *et al.*, 1997), hepatic and skin (Yu *et al.*, 1995) tumors *in-vitro* and *in-vivo*. Altshuler *et al.* (2013), found that the enantiomer (+)-citronellal, a major component of *Corymbia citriodora* and *Cymbopogon nardus* EOs, is also an effective microtubule-disrupting compound, similarly to better known microtubule-disrupting agents colchicine and vinblastine (Altshuler *et al.*, 2013).

Also β -caryophyllene (3.8%), caryophyllene oxide (10.2%), terpinen-4-ol (0.4%) and α -terpineol (0.3%) have also been demonstrated to act synergistically with other essential oil components to enhance cytotoxic activities (Wright *et al.*, 2007) therefore should be evaluate the synergy between these compounds and their own contribution to the final anticancer activity.

In this work and in the available literature, *M. officinalis* L. essential oil seems to have a potential as anticancer therapeutic agents. Altogether, our data

showed that this oil could be a natural source of anticancer compounds with antiproliferative and cytotoxic properties.

CONCLUSION

The results of our study suggest that the essential oil of *M. officinalis* L. has potential antiproliferative activity against several malignant cell lines (MOLT-4, MCF-7 and NCI-H460). The showed activities are differential and selective. The strongest antiproliferative activity of *M. officinalis* L. essential oil was observed against MOLT-4 cell line, resulted in >80% of growth inhibition in treated cells at the lowest applied dose. For all tested cell lines, a high level of cytotoxicity was observed. Solid tumor cell lines (MCF-7 and NCI-H 460) were more sensitive to higher concentrations of *M. officinalis* L. essential oil than hematological tumor cell lines (MOLT-4). Thus, from the above obtained results, we can observe that *M. officinalis* L. essential oil is showing a differential antiproliferative and cytotoxic effect at micromole concentrations, which are suited for further biological studies and optimization.

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