Cytotoxicity and ROS scavenging potential of *Teucrium montanum* L. methanolic extracts in HaCaT cells

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Abstract

Teucrium montanum L. (TM) is a perennial species of the Lamiaceae family, traditionally used in folk medicine for its diverse therapeutic properties. Although previous studies have explored the biological activity of TM extracts, their effects on skin cells have not been investigated. This study examined the cytotoxic and antioxidant effects of two methanolic extracts of *T. montanum* (TM1 and TM2), obtained from serpentinite and limestone, across a concentration range of 12.5–400 µg/mL on human keratinocyte (HaCaT) cells. Both extracts showed a concentration-dependent cytotoxic effect in the MTT assay, with no significant impact on cell survival up to 100 µg/mL. At higher concentrations (200 and 400 µg/mL), cell viability decreased significantly, with TM1 exhibiting greater cytotoxicity than TM2. Antioxidant potential was assessed by the H_2DCFDA assay, revealing that neither extract altered basal reactive oxygen species (ROS) levels, but higher concentrations increased ROS formation under H_2O_2-

induced oxidative stress. These results suggest that TM1 and TM2 are well tolerated at lower concentrations, but may induce oxidative stress and cytotoxicity at higher concentrations. The findings highlight the importance of dose optimisation for potential therapeutic or cosmetic applications of TM extracts and further phytochemical characterisation to identify the active constituents responsible for these effects.

Key words: *Teucrium montanum* L., methanolic extracts, MTT assay, H_2DCFDA assay, HaCaT cells

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Introduction

Oxidative stress occurs when the generation of reactive oxygen species (ROS) surpasses the body's antioxidant defences, disrupting the balance between oxidants and free radical scavenging systems (1). Excess ROS, including hydrogen peroxide, can oxidise proteins and lipids and cause DNA lesions, which can have both cytotoxic and genotoxic outcomes. Due to these effects, oxidative stress has been associated with the development of numerous pathological conditions (2). Plant-derived secondary metabolites are well-known for their antioxidant properties (3). Some of them, such as phenolic compounds, can directly counteract ROS or enhance the activity of endogenous antioxidant systems (4). By reducing oxidative DNA damage and protecting cellular structures from damage caused by radicals, they may help lower the risk of diseases linked to oxidative imbalance (5). Evaluating the biological activities of plant extracts is therefore crucial for identifying those with therapeutic value.

Serbia's diverse flora provides many medicinally valuable plants, including 41 species from the Lamiaceae family (5). Within this family, the genus *Teucrium* (germander) comprises more than 300 perennial species globally, of which 49 occur in Europe (6, 7). Reports suggest that members of this genus contain a wide range of compounds with significant biological potential (5, 8–10). *Teucrium montanum* L. (TM), commonly known as mountain germander (trava iva in Serbian), is among the seven *Teucrium* species identified in Serbia (11). Traditionally, TM has been applied in folk medicine both internally, such as in herbal teas, food preparations, or ethanolic extracts, and externally, including baths and tonics, for a variety of ailments (7, 12). Phytochemical investigations have revealed that TM contains numerous bioactive constituents. These include phenolic acids (hydroxylated benzoic and cinnamic acid derivatives), phenylethanoid glycosides (e.g., verbascoside, echinacoside), flavonoids along with their glycosides (such as luteolin, cirsiliol, rutin, apigenin, naringin, and quercetin derivatives), catechins, coumarins, diterpenoids (e.g., 19-acetylgnaphalin, montanins, teubotrin), and triterpenes (6, 7, 12). Experimental studies demonstrate that these phytochemicals play role in TM's wide range of biological activities, such as antioxidant, antibacterial, antifungal, pro-apoptotic, antiproliferative, and anti-inflammatory properties (6, 7, 13). Studies indicate that its methanolic extracts can selectively act against tumor cells without affecting resting or activated immune cells found in peripheral blood (14).

HaCaT cells, immortalised nontumorigenic monoclonal keratinocyte cell line, provide a practical, reproducible, and ethically acceptable *in vitro* system for examining the effects of plant extracts for topical application, particularly regarding cytotoxicity and antioxidant activity due to their normal phenotype and responses similar to normal human keratinocytes (15, 16). TM has been identified as a herbal source of bioactive compounds with potential applications as both a dietary supplement and therapeutic agent (12, 14). Certain species of the genus *Teucrium* have shown beneficial effects on the skin, such as anti-inflammatory, antioxidant, and anti-acne activities, as well as wound-healing properties (17, 18). However, there is no evidence regarding the effects of TM on skin cells. Studies have also confirmed that the biological activity of TM extracts is influenced by extraction techniques (7, 19, 20), but whether growing in ecologically different habitats also affects it has not yet been evaluated. Based on this, the present study aims to investigate two methanolic extracts of TM from different geological substrates, serpentinite and limestone, as potential sources of cell-protective and antioxidant agents.

Materials and methods

Plant Material and Extraction

The aerial parts of TM at the flowering stage were collected in July 2020 from Orovica Mountain in western Serbia (TM1; serpentinite; voucher deposited in the herbarium of the Faculty of Pharmacy, University of Belgrade – HFF 4302) and Vratna Gorge in eastern Serbia (TM2; limestone; voucher HFF 4301). The plant material was dried at room temperature. Samples of 73 g (TM1) and 77 g (TM2) were extracted with methanol (1:10; methanol 96% v/v) for 24 hours at room temperature to preserve potentially thermolabile compounds. Extraction was repeated twice, until the material was exhausted, and the solvent was evaporated under reduced pressure (BÜCHI Labortechnik R-100, Germany).

Cell culture

Human HaCaT keratinocytes were maintained in 25 cm^2 tissue culture flasks within a humidified incubator set to 37 °C with 5% CO_2. They were cultivated in a complete medium with Dulbecco's Modified Eagle Medium / Nutrient Mixture F-12 Ham (DMEM F 12, Biowest, Nuaillé, France), 10% fetal bovine serum (FCS, Gibco, Waltham, MA, USA) and 1% antimicrobial-antibiotic solution (Capricorn Scientific GmbH, Ebsdorfergrund, Germany). Upon attaining 70% confluence, treated with trypsin (0.25 % trypsin-EDTA solution, Institute for Virology, Vaccines and Serum "Torlak", Belgrade, Serbia), seeded in 96-well plates (1.5×10^4 cells/well) and left to adhere to the surface for 24 hours prior to treatment at 37 °C and 5% CO_2.

Preparation of the treatments

A stock solution of methanolic extracts (TM1 and TM2) was prepared in DMSO at a concentration of 100 mg/mL and stored at 4 °C. For the experiment, the final concentrations of each extract was prepared from the stock solution by mixing with fresh cell medium to obtain final concentrations of 12.5, 25, 50, 100, 200 and 400 µg/mL. These concentrations were used for further cell treatments.

Evaluation of cytotoxicity

Following overnight incubation, the medium was replaced after 24 hours and treatments were added at a total volume of 100 µL/well. After incubation with the treatments or solvent (control) at 37 °C for 24 hours, an MTT assay was performed. MTT reagent (thiazolyl blue tetrazolium bromide, 1 mg/mL, Sigma Aldrich, St. Louis, MO, USA) was added (10 µL per well), and the cells were kept in the dark at 37 °C for 2 hours to let the reaction take place. Subsequently, the purple formazan crystals were dissolved using sodium dodecyl sulfate (10% SDS in 0.01 M HCl, Sigma Aldrich, St. Louis, MO, USA). Finally, the absorbance was assessed at 570 nm using a microplate reader (Epoch, BioTek, USA) after the crystals were completely dissolved. The data were represented as percent viability relative to the control (100%). Mean values were calculated (n = 6).

H_2DCFDA assay (2’,7’-dichlorofluorescin diacetate)

After overnight incubation, the medium was changed and the extracts (TM1 and TM2) were added to the cells at the final concentrations (12.5–400 µg/mL) in complete medium (100 µL/well). After 24 hours, the treatments were removed and the cells were rinsed with PBS. The assay was then performed according to the manufacturer’s instructions. Using PBS as a diluent, 5 µM of the cell-permeable oxidation-sensitive probe H_2DCFDA (Merck Millipore, 2′,7′-dichlorofluorescin diacetate - CAS 4091-99-0 - Calbiochem) was added to the cells and left in the dark for 45 minutes. The cells were then washed with PBS and treated with PBS alone (control) or with 200 µM H_2O_2, which served to induce oxidative stress. After an incubation period of 1 hour and conversion of non-fluorescent H_2DCFDA to the highly fluorescent 2',7'-dichlorofluorescein (DCF), the generation of intracellular ROS in the cells was determined by measuring fluorescence on a fluorescence plate reader (Wallac 1420 Multilabel Counter Victor 3V) at excitation and emission wavelengths of 485 and 535, respectively. The data were expressed as relative fluorescence intensity, and the mean value was calculated (n = 6).

Statistical analysis

A one-way analysis of variance (ANOVA) utilizing Tukey post-hoc test was performed to evaluate the differences between the treatments and the control following the confirmation of data normality by using the Shapiro-Wilk test. The results are presented as mean + standard deviation (mean + S.D.). IC_{50} values were calculated using linear regression analysis. GraphPad Prism version 6.0 (GraphPad Software, Inc., La Jolla, CA, USA) was utilized for statistical evaluation, with $p < 0.05$ considered significant.

Results

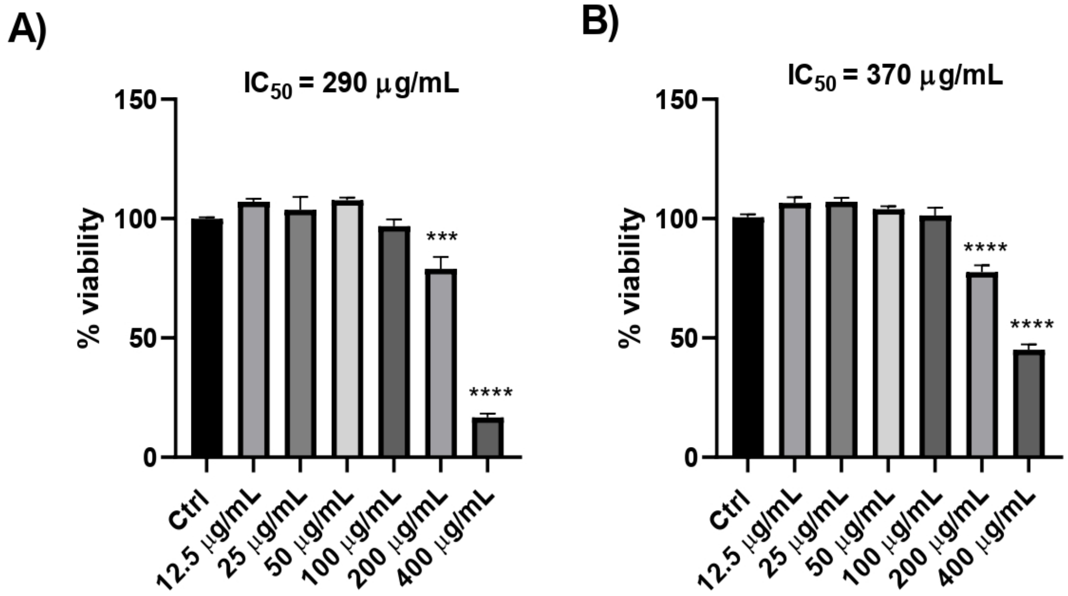

Figure 1. **Cytotoxicity of extracts A) TM1, B) TM2 in a range of concentrations (12.5, 25, 50, 100, 200 and 400 µg/mL) determined by MTT assay in HaCaT cells. The data are expressed as mean + S.D. relative to the unexposed control; *** p<0.001, **** p<0.0001 vs. control by one-way Analysis of Variance (ANOVA) with Tukey's multiple comparison post-hoc test**

Slika 1. **Citotoksičnost ekstrakata A) TM1, B) TM2 u rasponu koncentracija (12,5, 25, 50, 100, 200 i 400 µg/mL) određena MTT testom u HaCaT ćelijama. Podaci su izraženi kao srednja vrednost + S.D. u odnosu na neeksponiranu kontrolu; *** p<0,001, **** p<0,0001 u odnosu na kontrolu jednofaktorskom analizom varijanse (ANOVA) sa Tukijevim post-hok testom za višestruko poređenje**

Figure 1 shows the investigation of the cytotoxic potential of TM extracts in HaCaT cells. The incubation of the cells with TM1 and TM2 for 24 hours showed similar effects on cell viability. Both extracts caused no substantial alteration in cell viability at concentrations up to 100 µg/mL, compared to the unexposed control. Conversely, at concentrations of 200 and 400 µg/mL, both extracts led to a notable reduction in the percentage of viable cells compared to the control cells exposed to the medium alone. The extracts exhibited a concentration-dependent cytotoxic effect, with the most significant decrease in cell viability noted at the highest concentrations of TM1 and TM2. At 400 µg/mL, TM1 showed a more pronounced cytotoxic effect (84% reduction in cell viability) than TM2 (55% reduction in cell viability). The calculated IC_{50} values showed 290 µg/mL and 370 µg/mL, for TM1 and for TM2 respectively.

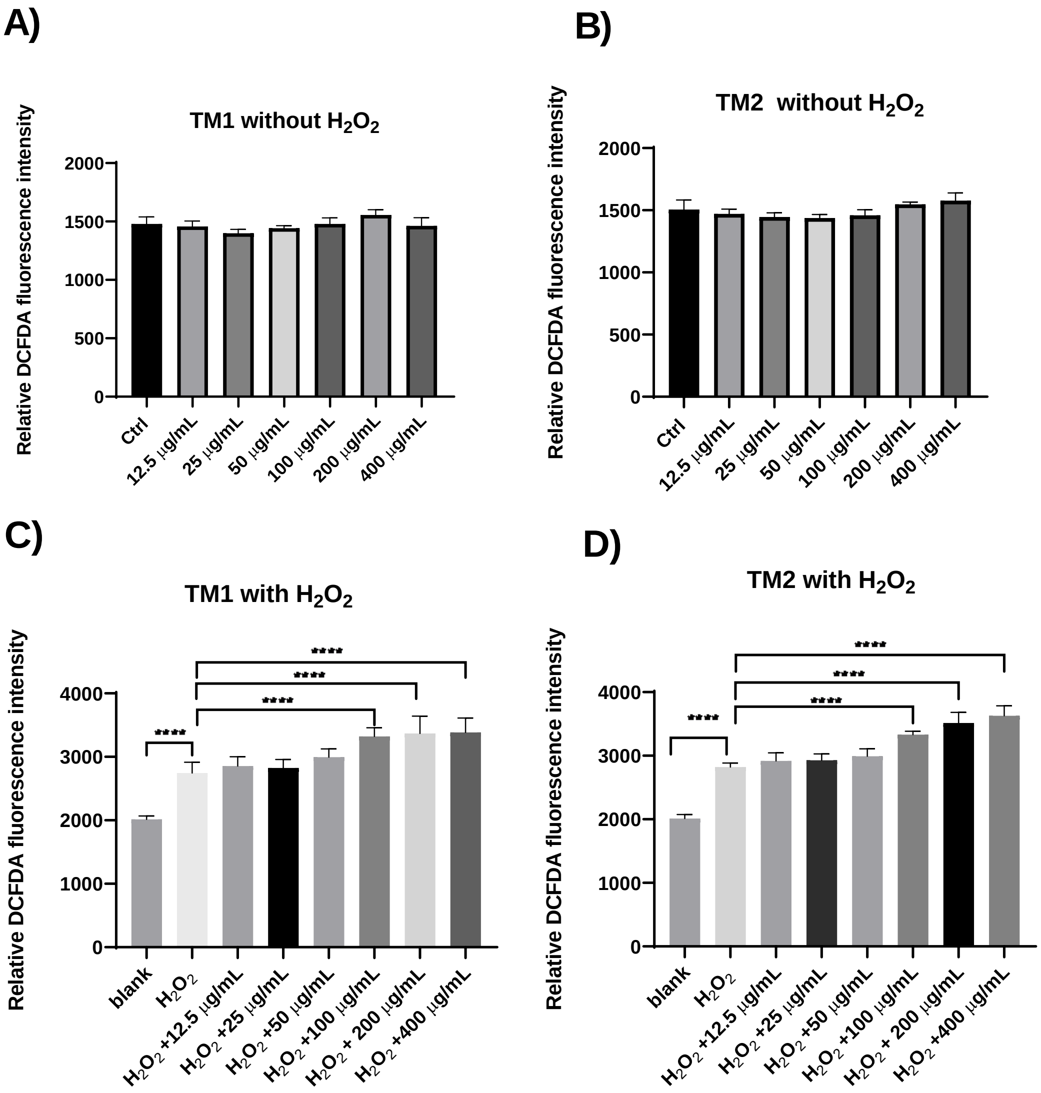

Figure 2. **Effect of 24h pre-incubation with extracts (TM1, TM2) in a range of concentrations (12.5, 25, 50, 100, 200 and 400 µg/mL) on the production of ROS in HaCaT cells without H_2O_2 (A, B) and after the exposure to H_2O_2 (C, D), determined by H_2DCFDA assay, expressed as relative fluorescence intensity. The data are expressed as mean + S.D.; **** $p<0.0001$ vs. control by one-way Analysis of Variance (ANOVA) with Tukey's multiple comparison post-hoc test**

Slika 2. **Efekat 24-časovne preinkubacije sa ekstraktima (TM1, TM2) u rasponu koncentracija (12,5, 25, 50, 100, 200 i 400 µg/mL) na nastanak ROS-a u HaCaT ćelijama bez H_2O_2 (A, B) i nakon izlaganja H_2O_2 (C, D), određeno H_2DCFDA testom, izraženo kao relativni intenzitet fluorescencije. Podaci su izraženi kao srednja vrednost + S.D.; **** $p<0,0001$ u odnosu na kontrolu jednofaktorskom analizom varijanse (ANOVA) sa Tukijevim post-hok testom za višestruko poređenje**

The effects of TM extracts on the production of ROS in HaCaT cells are shown on Figure 2. The analysis of the effects of the extracts on ROS levels prior to exposure to H_2O_2 revealed that TM1 (Figure 2A) and TM2 (Figure 2B) at selected concentrations did not cause any significant change in endogenous ROS production after 24 hours of incubation compared to the unexposed cells. After exposure to 200 µM H_2O_2 for 1 hour, the production of ROS in HaCaT cells was increased. The cells pre-incubated with TM1 (Figure 2C) and TM2 (Figure 2D) at concentrations 12.5, 25 and 50 µg/mL showed no change in ROS levels compared to the cells exposed only to H_2O_2. However, both extracts showed a significant concentration-dependent increase in ROS production in the cells pretreated with concentrations of 100, 200 and 400 µg/mL, indicating a pro-oxidant effect of TM1 and TM2 at high concentrations.

Discussion

Teucrium species are used in traditional medicine for the treatment of various diseases and conditions, which is why acquiring the knowledge about their safety and protective potential is very important. The *in vitro* cytotoxic and antioxidant activities of various extracts from *Teucrium* species have been investigated in different human cancer cell lines (14, 20, 21). Nevertheless, the effects of methanolic extracts of TM from ecologically distinct locations on healthy cells have not yet been examined. Environmental factors or extraction methods have been shown to significantly influence the content and activity of plant extracts (22, 23). In this study, the cytotoxic effects of TM1 and TM2 at different concentrations (12.5–400 µg/mL) on HaCaT cells were investigated using the MTT assay. The findings indicated a concentration-dependent cytotoxic effect of both extracts, whereby no significant changes in cell viability were observed at concentrations of up to 100 µg/mL. This indicates that both TM1 and TM2 are well tolerated by keratinocytes at lower concentrations and do not affect cell survival. Our findings are consistent with the results of Stanković et al. (14), showing that concentrations of up to 200 µg/mL of seven *Teucrium* extracts, including TM, had no cytotoxic effect on unstimulated peripheral blood mononuclear cells (PBMCs). The higher concentrations of TM1 and TM2 tested in this study (200 and 400 µg/mL) significantly reduced cell viability, indicating the onset of cytotoxic effects. This is also consistent with Stanković et al. (14), where TM extract at a concentration of 200 µg/mL reduced the survival rate of non-stimulated and PHA-stimulated PBMCs by up to 10% and 20%, respectively. Furthermore, the methanolic extract of *T. oliverianum* demonstrated safety on HaCaT cells, with cell viability remaining above 80% at concentrations up to 250 µg/mL and an IC_{50} of 676.2 ± 0.111 µg/mL (17). The results of our study show that TM1 reduced cell viability by 84%, while TM2 caused a 55% reduction at the highest concentration tested. Together with the IC_{50} values of 290 µg/mL for TM1 and 370 µg/mL for TM2, this indicates that TM1 has a stronger cytotoxic effect on HaCaT cells at higher concentrations. Both extracts exhibit relatively low cytotoxicity compared with some other *Teucrium* species, such as *T. polium* and *T. persicum*, which demonstrated markedly stronger cytotoxicity against cancer cell lines ($IC_{50} \approx 30$ µg/mL and up to 65.9 µg/mL, respectively), reflecting selective anticancer activity rather than general cytotoxicity (24, 25). This difference in response between TM1 and TM2 is likely due to the distinct qualitative and quantitative phytochemical compositions or bioactive constituents of the two extracts, warranting their further phytochemical characterisation. It is confirmed that the extraction technique and growing conditions greatly influence the composition and degree of biological activity of TM extracts and essential oils (7, 26, 27).

In terms of antioxidant activity, various TM extracts have been shown to neutralize DPPH radicals, reduce nitric oxide concentration, and exhibit remarkable scavenging activity for hydroxyl radicals, which increases with concentration (7, 20, 26). TM extracts are a significant source of phenolic compounds exhibiting strong antioxidant properties (9). This is mainly due to the redox characteristics of these compounds, which enable them to scavenge free radicals, reduce their generation, and act as chelators for transition metals, providing an important protective effect (5, 20). ROS produced in skin cells are a significant contributor to the ageing process of the skin. Plant extracts, being a rich source of antioxidants, can decrease intracellular oxidative stress, improving the skin's ability to slow down the ageing process and playing an important role in wound healing (28–30). In our study, the influence of TM1 and TM2 on the intracellular production of ROS in HaCaT cells was analysed using the H_2DCFDA assay. The extracts did not alter endogenous ROS production at any of the tested concentrations when administered alone, suggesting that they are not inherently prooxidant under homeostatic conditions. However, a different pattern was observed when the cells were pretreated with the extracts before being exposed to H_2O_2-induced oxidative stress. At lower concentrations (12.5, 25 and 50 µg/mL), neither TM1 nor TM2 had a significant effect on ROS levels, indicating a lack of protective antioxidant activity under these conditions. Conversely, higher concentrations (100, 200 and 400 µg/mL) led to a significant increase in ROS formation in the presence of H_2O_2, indicating a prooxidant effect. This paradoxical concentration-dependent behaviour suggests that although TM1 and TM2 were relatively inert at low concentrations, at higher concentrations they can exacerbate oxidative stress, especially in an already stressed cellular environment. Such biphasic effects are common in phytochemicals and may be due to redox-active secondary metabolites that switch from antioxidant to prooxidant behaviour depending on the dose and cellular environment (31, 32). Despite providing novel insights into the cytotoxic and redox-related effects of TM, several limitations of this study should be acknowledged. They include the use of *in vitro* models only, reliance on a single extraction method, and small environmental variability of the samples. These factors may restrict the broader applicability of the results. Future research should incorporate larger sample sets, alternative extraction approaches, detailed phytochemical characterization, and *in vivo* studies to better clarify the safety profile, mechanisms of action, and therapeutic relevance of TM extracts.

Conclusion

In summary, TM1 and TM2 extracts demonstrate concentration-dependent cytotoxic and ROS generative effects on HaCaT cells. Both extracts are non-toxic at lower concentrations, but higher concentrations significantly reduce cell viability and increase ROS production, especially under conditions of oxidative stress. TM1 shows a more potent cytotoxic and pro-oxidative profile than TM2. These findings suggest that careful dose consideration is essential for any future therapeutic or cosmetic application of these extracts. Further studies should focus on identifying the active components responsible for these effects and explore possible benefits at sub-toxic concentrations.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

D.T.: Conceptualization, Data interpretation, Validation, Writing – original draft; **A.P.**: Methodology, Investigation, Formal analysis, Visualization, Writing – review & editing; **M.Z.:** Investigation, Formal analysis, Data curation; **B.S.P.**: Data interpretation, Writing – review & editing; **L.Ž.**: Methodology, Writing – review & editing; **M.J.K.**: Validation, Writing – review & editing; **M.M.**: Conceptualization, Data interpretation, Supervision, Writing – review & editing.

References

1. Gupta RK, Patel AK, Shah N, Choudhary AK, Jha UK, Yadav UC, et al. Oxidative Stress and Antioxidants in Disease and Cancer: A Review. Asian Pacific J Cancer Prev. 2014;15(11):4405–9.
2. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, et al. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018;13:757–72.
3. Marčetić M, Arsenijević J. Antioxidant activity of plant secondary metabolites. Arh Farm. 2023;73(4):264–77.
4. Lin D, Xiao M, Zhao J, Li Z, Xing B, Li X, et al. An Overview of Plant Phenolic Compounds and Their Importance in Human Nutrition and Management of Type 2 Diabetes. Molecules. 2016;21(10):1374.
5. Oalđe MM, Kolarević SM, Živković JC, Vuković-Gačić BS, Jovanović Marić JM, Kračun Kolarević MJ, et al. The impact of different extracts of six Lamiaceae species on deleterious effects of oxidative stress assessed in acellular, prokaryotic and eukaryotic models in vitro. Saudi Pharm J. 2020;28(12):1592–604.

6. Li JJ, Ma S, Wang Y, Wang M, Li M, Gao C, et al. Teucrium montanum extract drives effector and memory differentiation of CD8+ T cells. Biomed Res Ther. 2023;10(11):6023–34.
7. Sailović P, Odžaković B, Bodroža D, Vulić J, Čanadanović-Brunet J, Zvezdanović J, et al. Polyphenolic Composition and Antimicrobial, Antioxidant, Anti-Inflammatory, and Antihyperglycemic Activity of Different Extracts of Teucrium montanum from Ozren Mountain. Antibiotics. 2024;13(4):358.
8. Katalinic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem. 2006;94(4):550–7.
9. Seremet D, Vojvodic Cebin A, Mandura A, Komes D. Valorisation of Teucrium montanum as a Source of Valuable Natural Compounds: Bioactive Content, Antimicrobial and Biological Activity – A Review. Pharmacogn Rev. 2021;15(30):191–8.
10. Bektasevic M, Jurin M, Roje M, Politeo O. Phytochemical Profile, Antioxidant Activity and Cholinesterase Inhibition Potential of Essential Oil and Extracts of Teucrium montanum from Bosnia and Herzegovina. Separations. 2023;10(8):421.
11. Milošević-Djordjević O, Stošić I, Stanković M, Grujičić D. Comparative study of genotoxicity and antimutagenicity of methanolic extracts from Teucrium chamaedrys and Teucrium montanum in human lymphocytes using micronucleus assay. Cytotechnology. 2013;65(5):863–9.
12. Aćimović M, Stanković Jeremić J, Miljković A, Rat M, Lončar B. Screening of Volatile Compounds, Traditional and Modern Phytotherapy Approaches of Selected Non-Aromatic Medicinal Plants (Lamiaceae, Lamioideae) from Rtanj Mountain, Eastern Serbia. Molecules. 2023;28(12):4611.
13. Bufan B, Marčetić M, Djuretić J, Ćuruvija I, Blagojević V, Božić DD, et al. Evaluation of the Anti-Inflammatory/Immunomodulatory Effect of Teucrium montanum L. Extract in Collagen-Induced Arthritis in Rats. Biology. 2024;13(10):818.
14. Stanković MS, Mitrović TL, Matić IZ, Topuzović MD, Stamenković SM. New Values of Teucrium species: in Vitro Study of Cytotoxic Activities of Secondary Metabolites. Not Bot Horti Agrobot Cluj-Napoca. 2015;43(1):41–6.
15. Micallef L, Belaubre F, Pinon A, Jayat-Vignoles C, Delage C, Charveron M, et al. Effects of extracellular calcium on the growth-differentiation switch in immortalized keratinocyte HaCaT cells compared with normal human keratinocytes. Exp Dermatol. 2009;18(2):143–51.
16. López-García J, Lehocký M, Humpolíček P, Sáha P. HaCaT Keratinocytes Response on Antimicrobial Atelocollagen Substrates: Extent of Cytotoxicity, Cell Viability and Proliferation. J Funct Biomater. 2014;5(2):43–57.
17. Al-Ghanayem AA. In-vitro anti-acne activity of Teucrium oliverianum methanolic extract against Cutibacterium acnes. Front Pharmacol. 2024;15:1388625.
18. Chabane S, Boudjelal A, Keller M, Doubakh S, Potterat O. Teucrium polium - wound healing potential, toxicity and polyphenolic profile. South African J Bot. 2021;137:228–35.
19. Vujanović M, Zengin G, Đurović S, Mašković P, Cvetanović A, Radojković M. Biological activity of extracts of traditional wild medicinal plants from the Balkan Peninsula. South African J Bot. 2019;120:213–8.
20. Stankovic MS, Curcic MG, Zizic JB, Topuzovic MD, Solujic SR, Markovic SD. Teucrium Plant Species as Natural Sources of Novel Anticancer Compounds: Antiproliferative, Proapoptotic and Antioxidant Properties. Int J Mol Sci. 2011;12(7):4190–205.

21. Zaric M, Zivkovic-Zaric R, Mitrovic M, Nikolic I, Canovic P, Milosavljevic Z, et al. Teucrium polium induces apoptosis in peripheral blood lymphocytes isolated from human chronic lymphocytic leukemia. Vojnosanit Pregl. 2020;77(12):1252–9.
22. Elsharkawy ER, Alghanem SM, Elmorsy E. Effect of habitat variations on the chemical composition, antioxidant, and antimicrobial activities of Achillea fragrantissima (Forssk) Sch. Bip. Biotechnol Reports. 2021;29:e00581.
23. Rawat P, Dasila K, Singh M, Kuniyal JC. Influence of environmental factors on phytochemical compositions and antioxidant activity of Juniperus communis L. Discov Environ. 2025;3(1):11.
24. Bsharat O, Salama Y, Saed E, Al-Hajj N, Al-Maharik N. Assessing Teucrium polium L. from chemical profiling to antioxidant, anticancer, α-amylase, and lipase activities. Sci Rep. 2025;15(1):34371.
25. Hajipour P, Eizadifard F, Tafrihi M. Chemical Constituents, Antioxidant and Cytotoxic Potential of Chloroform and Ethyl Acetate Extracts of Teucrium persicum. Jentashapir J Cell Mol Biol. 2022;13(2):e128492.
26. Čanadanović-Brunet JM, Djilas SM, Ćetković GS, Tumbas VT, Mandić AI, Čanadanović VM. Antioxidant activities of different Teucrium montanum L. Extracts. Int J Food Sci Technol. 2006;41(6):667–73.
27. Zlatić N, Mihailović V, Lješević M, Beškoski V, Stanković M. Geological substrate-related variability of Teucrium montanum L. (Lamiaceae) essential oil. Biochem Syst Ecol. 2022;100:104372.
28. He X, Gao X, Guo Y, Xie W. Research Progress on Bioactive Factors against Skin Aging. Int J Mol Sci. 2024;25(7):3797.
29. Lee SG, Ham S, Lee J, Jang Y, Suk J, Lee YI, et al. Evaluation of the anti-aging effects of Zinc-α2-glycoprotein peptide in clinical and in vitro study. Ski Res Technol. 2024;30(3):e13609.
30. Hussen NH, Abdulla SK, Ali NM, Ahmed VA, Hasan AH, Qadir EE. Role of antioxidants in skin aging and the molecular mechanism of ROS: A comprehensive review. Asp Mol Med. 2025;5:100063.
31. Xi X, Wang J, Qin Y, You Y, Huang W, Zhan J. The Biphasic Effect of Flavonoids on Oxidative Stress and Cell Proliferation in Breast Cancer Cells. Antioxidants. 2022;11(4):622.
32. Kang SG, Lee GB, Vinayagam R, Do GS, Oh SY, Yang SJ, et al. Anti-Inflammatory, Antioxidative, and Nitric Oxide-Scavenging Activities of a Quercetin Nanosuspension with Polyethylene Glycol in LPS-Induced RAW 264.7 Macrophages. Molecules. 2022;27(21):7432.

Ispitivanje citotoksičnosti i sposobnosti uklanjanja ROS-a metanolnih ekstrakata *Teucrium montanum* L. u HaCaT ćelijama

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Kratak sadržaj

Teucrium montanum L. (TM) je višegodišnja vrsta iz porodice Lamiaceae, koja se tradicionalno koristi u narodnoj medicini zbog svojih raznovrsnih terapeutskih svojstava. Iako su prethodne studije ispitivale biološku aktivnost TM ekstrakata, njihovi efekti na ćelije kože nisu istraženi. U ovoj studiji su ispitani citotoksični i antioksidativni efekti dva metanolna ekstrakta *T. montanum* (TM1 i TM2), sa serpentinita i krečnjaka, u opsegu koncentracija od 12,5–400 µg/mL na ćelije humanih keratinocita (HaCaT). Oba ekstrakta su pokazala koncentraciono zavisan citotoksični efekat u MTT testu, bez uticaja na preživljavanje ćelija do koncentracije od 100 µg/mL. Pri višim koncentracijama (200 i 400 µg/mL), vijabilnost ćelija je značajno smanjena, pri čemu je TM1 pokazao veću citotoksičnost od TM2. Antioksidativni potencijal je procenjen H_2DCFDA testom, koji je pokazao da nijedan ekstrakt nije promenio bazalne nivoe reaktivnih vrsta kiseonika (ROS), ali su više koncentracije ekstrakta povećale nivo ROS-a pod oksidativnim stresom izazvanim vodonik-peroksidom. Ovi rezultati ukazuju na to da ćelije tolerišu TM1 i TM2 u nižim koncentracijama, ali da više koncentracije mogu izazvati oksidativni stres i biti citotoksične. Rezultati ukazuju na važnost optimizacije doze pri potencijalnoj terapeutskoj ili kozmetičkoj primeni ekstrakata TM kao i na potrebu za daljom fitohemijskom karakterizacijom kako bi se identifikovali njihovi aktivni sastojci.

Ključne reči: *Teucrium montanum* L., metanolni ekstrakti, MTT test, H_2DCFDA test, HaCaT ćelije