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ORIGINAL ARTICLE / ÖZGÜN MAKALE

INVESTIGATION OF CHEMICAL COMPOSITION AND ANTICANCER ACTIVITY OF *STACHYS OFFICINALIS* (L.) TREVISAN SUBSP. *BALCANICA* (P.W. BALL) R. BHATTACHARJEE ESSENTIAL OIL

STACHYS OFFICINALIS (L.) TREVISAN SUBSP. BALCANICA (P.W. BALL) R. BHATTACHARJEE UÇUCU YAĞININ KİMYASAL BİLEŞİMİ VE ANTİKANSER AKTİVİTESİNİN ARAŞTIRILMASI

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ABSTRACT

Objective: *This study focuses on Stachys officinalis (L.) Trevisan subsp. balcanica (P.W. Ball) R. Bhattacharjee aiming to elucidate its essential oil's chemical composition and evaluate its potential in cancer treatment.*
Material and Method: *In this study, plant specimens were collected from Bolu province, Turkey. The collected plant material was authenticated and prepared according to herbarium techniques. The essential oil was obtained by hydrodistillation using a Clevenger apparatus. Thin layer chromatography was employed for preliminary analysis of the essential oil's compound groups. For further chemical component separation and identification, gas chromatography and gas chromatography-mass spectrometry were used. Antiproliferative and cytotoxic activities were assessed via the MTT assay on Caco-2 colon cancer cells and Vero normal cells.*
Result and Discussion: *Phytochemical analysis of S. officinalis subsp. balcanica essential oil (SOBEO) identified 32 compounds, representing 82.7% of the oil, with α-selinene (12.1%), β-selinene (11.4%), and β-caryophyllene (7.2%) as the major constituents. Several components uncommon in other Stachys species suggest chemovarietal diversity. SOBEO (5 µg/ml) inhibited Caco-2 colon cancer cell growth by 41.56% at 48 h, while showing no significant cytotoxicity toward Vero cells. This study provides the first comprehensive characterization of the chemical profile and anticancer potential of this subspecies, addressing a key gap in current knowledge.*
Keywords: *Anticancer activity, essential oils, gas chromatography, Stachys officinalis subsp. balcanica*

ÖZ

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Amaç: *Bu çalışma, Stachys officinalis (L.) Trevisan subsp. balcanica (P.W. Ball) R. Bhattacharjee bitkisinin uçucu yağının fitokimyasal bileşimini belirlemek ve kolon kanserine karşı potansiyel antiproliferatif ve sitotoksik etkilerini değerlendirmek amacıyla gerçekleştirilmiştir.*

Gereç ve Yöntem: *Bitki örnekleri Türkiye'nin Bolu ilinden toplanmış, teşhis edilmiş ve herbaryum tekniklerine uygun şekilde saklanmıştır. Uçucu yağ, Clevenger düzeneği kullanılarak hidrodistilasyon yöntemiyle elde edilmiştir. Bileşikler önce ince tabaka kromatografisi ile ön analize tabi tutulmuş, ardından gaz kromatografisi ve gaz kromatografisi-kütle spektrometresi ile tanımlanmıştır. Antiproliferatif ve sitotoksik aktiviteler, Caco-2 kolon kanseri hücreleri ve Vero normal hücreleri üzerinde MTT testi ile değerlendirilmiştir.*

Sonuç ve Tartışma: *Uçucu yağda toplam %82.7'yi oluşturan 32 bileşen belirlenmiştir. Başlıca bileşenler α-selinen (%12.1), β-selinen (%11.4) ve β-karyofillen (%7.2) olarak tespit edilmiştir. Caco-2 hücrelerinde 5 µg/ml SOBEO uygulaması, 48 saat sonunda %41.56 oranında büyüme inhibisyonu sağlamıştır. Vero hücrelerinde anlamlı düzeyde toksisite gözlenmemiştir. Bazı bileşenlerin diğer Stachys türlerinde tipik olarak bulunmaması, tür içi kemovaryetal çeşitliliğe işaret etmiştir. S. officinalis subsp. balcanica uçucu yağının seçici antikanser aktivite gösterdiği ve kimyasal bileşim açısından özgün özellikler taşıdığı belirlenmiştir. Bulgular, bu alttürün hem fitokimyasal yapısına hem de biyolojik aktivitesine yönelik ilk kapsamlı verileri sunmaktadır. Konu ile ilgili daha ileri çalışmalar, aktif bileşenlerin izolasyonu ve mekanizma temelli aktivite çalışmaları ile desteklenmelidir.*

Anahtar Kelimeler: *Antikanser aktivite, gaz kromatografisi, Stachys officinalis subsp. balcanica, uçucu yağlar*

INTRODUCTION

Cancer is a chronic disease characterized by the formation of tissue masses called tumors, which arise from irregular and uncontrolled proliferation and spread of cells within these masses. Based on the 2022 data from the Global Cancer Research Project (GLOBOCAN), there were a total of 19.7 million individuals diagnosed with cancer globally, resulting in 9.7 million deaths linked to cancer. Due to the growing human population and the presence of risk factors, including smoking, obesity, and physical inactivity, it is expected that cancer-related mortality will increase worldwide in the next decade.

The use of plants for therapeutic purposes in treating many diseases can be traced back to over 60,000 years ago. According to the World Health Organization (WHO), natural resources and herbal medicines are beneficial in many areas of primary healthcare services for 80% of the world's population (4 billion people) [1]. One significant area of treatment using herbal products is aromatherapy. Aromatherapy, a complementary and supportive treatment method, utilizes biologically active essential oils in appropriate mixtures, dosages, and durations to treat symptomatic diseases [2]. Essential oils or substances in various parts of aromatic plants (flowers, bark, branches, fruit, seed, rhizome, leaf) are used in aromatherapy [3]. Essential oils, found in natural products and used for medical purposes in aromatherapy, frequently feature in studies for cancer treatment due to their low side effect profiles and different mechanisms of action as angiogenesis inhibitors. The search for novel anticancer compounds derived from natural sources has garnered considerable scientific and commercial attention, as indicated by the growing body of research focused on treating this disease [4]. Ongoing efforts persist in preventing and treating the disease, with an increasing demand for novel substances derived from natural sources [5]. In this context, significant studies have determined essential oils' antiproliferative and cytotoxic activities [6,7,8].

Lamiaceae family, rich in essential oil-bearing plants, includes annual or perennial herbaceous, shrubby, and rarely woody plants. It is an important family that carries labiate-type secretory trichomes and contains essential oils [9]. Genus *Stachys* L., one of the broadest taxa within the Lamiaceae family, typically spreads in temperate and tropical regions and includes annual or perennial herbaceous and small shrub species. Represented worldwide by 375 species and 435 taxa, the *Stachys* genus is phytochemically significant, with over 200 compounds isolated from this genus, including diterpenes, triterpenes, flavonoids, iridoids, phenolic acids, phenylethanoid glycosides, lignans, and essential oils. Globally, species of the *Stachys* genus are evaluated for medical and economic purposes due to their rich phytochemical content. Many *Stachys* species are grown for medicinal use, the food business, the cosmetic industry, and decorative uses because to the growing demand for natural goods [10]. Literature

on *Stachys* taxa highlights studies related to anticancer, antioxidant, antiproliferative, anti-inflammatory, anxiolytic, and antimutagenic activities. Furthermore, *Stachys* species have long been employed in traditional medicine. From an ethnobotanical perspective, their use in treating liver disorders, epilepsy, common colds, stomach ulcers and their efficacy on tumors is particularly significant for their anticancer activity potential [11,12].

Some studies have been conducted on different species within the *Stachys* genus that are not covered in this work, focusing on the phytochemical analysis of essential oils.

Considering the outcomes of these studies, the *Stachys* species possess potential for therapeutic use and the development of standardized medical products required for treatment. This introduces a new area of research focused on the taxon *Stachys officinalis* (L.) Trevisan subsp. *balcanica* (P.W. Ball) R. Bhattacharjee. *S. officinalis* subsp. *balcanica* essential oil is used in traditional medicine for the treatment of certain diseases and cancer. In order to put this traditional application on a scientific basis, utilizing a Clevenger equipment in accordance with the European Pharmacopoeia-registered procedure, the plant's essential oil was distilled. The anticancer effect of the essential oil was identified using antiproliferative and cytotoxic activity tests based on the *in vitro* MTT method. The study focused on obtaining the essential oil of *S. officinalis* subsp. *balcanica* (SOBEO) and elucidating its phytochemical content through GC-MS analysis. This study aimed to evaluate the potential use of SOBEO in cancer treatment. In this direction, the chemical composition of the essential oil was illuminated by gas chromatography for the first time. Since there has been no previous study on the essential oil of this taxon, it emerges as a subject open to further exploration in further research.

MATERIAL AND METHOD

Plant Material

S. officinalis subsp. *balcanica* specimens were collected in July 2020, from Bolu province, Turkey. The specific collection locality was Abant Lake entrance on the left, with coordinates 40º 36' 20.3632'' N, 31º 16' 37.2126" E and an altitude of 1330 meters. The collected plant samples were authenticated by Prof. Dr. Hayri Duman from Gazi University Faculty of Science, Department of Biology and the voucher specimen of *S. officinalis* subsp. *balcanica* was prepared and cataloged under the accession number AEF 29952. Subsequently, the plant material was appropriately dried and ground into powder.

Extraction of Essential Oil

The hydrodistillation of *S. officinalis* subsp. *balcanica* essential oils (SOBEO) was conducted by using a Clevenger apparatus, endorsed by the European Pharmacopoeia. This process began by using 200 grams of powdered aerial parts of the plant in water, filling up to one liter in a 5-liter distillation flask, approximately two-thirds of its capacity. The setup was then heated using a mantle heater, maintaining a temperature range of 80-100°C, ensuring the continuous flow of cold water through the condenser to facilitate efficient condensation of the vaporized essential oil. Hydrodistillation was conducted until no more essential oil could be obtained from the powdered plant material (over a period of 4 hours). The collected distillate was transferred into a pre-weighed vial using a syringe and washed over with n-hexane for further purification and quantification of the yield based on weight. Before conducting anti-cancer and phytochemical activity analyses, the essential oil was stored at 4°C to preserve its chemical integrity. The yield of the essential oil obtained was calculated based on the following equation:

$$\%yield = \frac{weight\ of\ the\ essential\ oil}{weight\ of\ the\ sample} \times 100$$

Thin Layer Chromatography

SOBEO was subjected to chromatographic analysis using Thin Layer Chromatography (TLC) with appropriate and varied solvent systems for a preliminary evaluation of its active compound groups.

The analysis employed a solvent system of toluene: ethyl acetate (93:7) alongside silica gel 60 F254 (Merck, 1.0554, 20x20) coated plates, aiming to discern the distinct compounds present within the essential oil. Following TLC, the chromatograms were initially examined under UV light at 366 nm and 254 nm to identify compounds based on their UV absorption. To detect substances that did not exhibit UV absorption, vanillin-H_2SO_4 mixture was utilized as a reagent. The preparation of this reagent involved dissolving 0.5 grams of vanillin in 50 ml of ethanol (Solution 1), followed by mixing 5 ml of concentrated H_2SO_4 with 50 ml of ethanol in a separate container (Solution 2). After the UV inspection of the plates, solutions 1 and 2 were sequentially sprayed onto them. The plates were then incubated in an oven for 10 minutes at 110ºC, allowing the spots to become distinct and visible for analysis under daylight.

GC/MS Analyses

Following the preliminary analysis with thin layer chromatography (TLC), the separation and identification of the chemical components of SOBEO were further pursued using Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS), illuminating the phytochemical structure of the oil. The analyses of SOBEO were conducted using a concurrent GC and GC/MS system. The GC analysis was performed on an Agilent 7890B GC System equipped with an Agilent HP-Innowax column (60 m x 0.25 mm i.d. x 0.25 µm film thickness). The oven temperature was initially held at 60°C for 10 minutes, then ramped to 220°C at 4°C/min and held for 10 minutes, followed by a final increase to 240°C at 1°C/min for a total runtime of 80 minutes; injector and detector temperatures were maintained at 250°C. Helium was used as the carrier gas at a 0.7 ml/min flow rate. A sample prepared in hexane (10% w/w) was injected into the system with a 40:1 split ratio at a volume of 1 µL. The separated compounds were detected using a Flame Ionization Detector (FID), and the relative percentages of the compounds were determined.

The GC/MS analysis was carried out using an Agilent 7890B GC system coupled with a 5977B Mass Selective Detector, employing the same Agilent HP-Innowax column. The temperature program for the GC/MS was identical to that of the GC analysis; however, the injection and ion source temperatures were set at 250°C and 230°C, respectively. Helium served as the carrier gas with a 0.7 ml/min flow rate. Mass spectra were acquired across a mass range of 35-450 m/z, with an ionization voltage of 70 eV utilized within the system. Individual mass spectra for the separated compounds were obtained through the GC/MS column, and identification processes were conducted using the Wiley 9-Nist 11 Mass Spectral Database.

MTT Analyses

SOBEO, whose phytochemical structure has been elucidated, was examined for its antiproliferative and cytotoxic activities using *in vitro* experimental models. Caco-2 colon cancer cells and Vero cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) high glucose medium (Gibco) supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin/streptomycin. Subsequently, the cell lines were incubated with 5% CO_2 and 95% humidity at 37°C. Caco-2 colon cancer cells and Vero normal cells were seeded into 96-well plates at a density of $1x10^4$ cells per well. After 24 hours, the cells were treated with samples of different polarities. The cells were exposed to varying concentrations of the samples (5, 10, 50, and 100 µg) for 24, 48, and 72 hours to determine the antiproliferative and cytotoxic effect profiles at these dosages. The medium was discarded after each treatment, and 20 µL of 5 mg/ml MTT dye was added to each well. The cells were then incubated for approximately 4 hours at 37°C in a 5% CO_2 incubator. The MTT solution was subsequently removed, and 100 µL of dimethyl sulfoxide (DMSO) was added to dissolve the insoluble formazan crystals. The plates were agitated for 15 minutes at room temperature to ensure complete dissolution. The level of the colored formazan derivative was measured at 590 nm using a microplate reader. Experiments were conducted in triplicate, and results were analyzed and visualized by GraphPad Prism 9.0 software. Combined evaluation of phytochemical analysis and *in vitro* cytotoxicity studies allowed for an assessment of the anticancer efficacy of SOBEO, providing scientific validation for the plant's traditional use in this area through contemporary *in vitro* techniques. The percentage of cell viability was calculated using the following formula:

$$\%viability = \frac{mean\ of\ the\ optical\ density\ of\ treated\ cells}{mean\ of\ the\ optical\ density\ of\ control\ cells} \times 100$$

$$\%cytotoxicity = Percent\ of\ viability \times 100$$

RESULT AND DISCUSSION

Chemical Composition of the Essential Oil

Stachys genus is distinguished by many species renowned for the rich chemical composition of their essential oils, upon which numerous phytochemical studies have been conducted. This study delves into the phytochemical analysis of SOBEO utilizing TLC, and GC-MS for its comprehensive evaluation.

The chromatographic profiling of SOBEO commenced with TLC analyses, employing a toluene: ethyl acetate (93:7) solvent system, chosen for its superior separation capabilities. The chromatograms were examined under UV light at 264 nm and 366 nm, with vanillin-H_2SO_4 reagent subsequently applied to enhance the visibility of components not readily observed under UV. TLC chromatograms of the essential oil show that the terpenoid structures found in the content of the essential oil, in red-brown and purple color following the application of vanillin-H_2SO_4.

The yield of SOBEO obtained from the hydrodistillation of powdered *S. officinalis* subsp. *balcanica* was 0.05% (v/w). GC/MS facilitated the identification of the essential oil's components, whereas GC determined their relative percentages. The composition of SOBEO is given in Table 1 (PL. In total, 32 compounds were detected, accounting for 82.7% of the oil composition, with five constituents remaining unidentified due to the absence of matching entries in the current dataset. The most abundant compounds in chemical composition of SOBEO were identified as α-selinene (12.1%), β-selinene (11.4%), and β-caryophyllene (7.2%).

Phytochemical studies on *Stachys* species reveal that essential oils constitute significant secondary metabolites of the species. The main components identified in the essential oils of *Stachys* species are monoterpenes and sesquiterpenes. Essential oil obtained from the aerial parts of *S. officinalis* collected from Serbia has shown sesquiterpene hydrocarbons (69.1%) as the leading component group. Germacranes, cadinanes, related sesquiterpenoids, and caryophyllenes represent the most abundant primary compounds [13]. A study by Goren and colleagues in 2011 evaluated the chemical profiles of essential oils from 22 *Stachys* species, reporting 39 compounds that constitute 70.5-97.8% of the total oil composition, with germacrene-D (2.9-45.3%), *β*-caryophyllene (2.3-62.3%), caryophyllene oxide (12.8%), spathulenol (7.8%), and *α*-cadinene (1.4-8.5%) identified as primary components [14]. Another study investigated the chemical composition of essential oils *from S. sylvatica* grown in Kosovo, α-pinene, *β*-pinene, and germacrene-D were identified as main components in the leaves and flowers. In contrast, essential oil from *S. sylvatica* collected in Turkey showed *α*-pinene, *β*-caryophyllene and germacrene-D; however, *β*-pinene was not detected [15].

Numerous studies have explored the chemical substance of *Stachys* species, revealing a rich diversity of essential oil components. The SOBEO demonstrated remarkable diversity when compared with the parent compounds of the essential oils of other *Stachys* species. Unlike those typically reported in the literature for *Stachys* species—predominantly germacrene-D, *α*-pinene, *β*-pinene, and *β*-caryophyllene— major part of the main constituents were not found in *S. officinalis* subsp. *balcanica* oil. Instead, *α*-elinene (12.1%), *β*-selinene (11.4%), and *β*-caryophyllene (7.2%) were the most abundant compounds in SOBEO. The absence of typical primary compounds in the current study suggests that observed differences may be attributed to the chemo varietal diversity within the plant species. *β*-caryophyllene has been identified as a major metabolite in the essential oils of various *Stachys* species in other research [14,15]. However, *α*-selinene and *β*-selinene have not been previously reported as significant components in the essential oil of *Stachys* species. It is acknowledged that a phytochemical profile determines bioactivity; therefore, an extensive evaluation of the phytochemistry of natural products is crucial for a comprehensive understanding of their therapeutic potential.

Table 1. Chemical composition of SOBEO. * ≥ % 0.5

No	Compounds*	Relative percentage (%)
1	*α*-cubebene	0.5
2	trans-*α*-bergamotene	1.3
3	*β*-caryophyllene	7.2
4	(E)-β-Farnesene	0.5
5	*α*-humulene	1.9
6	*γ*-muurolene	2.5
7	4,11-selinadien	0.9
8	*β*-bisabolene	0.6
9	*α*-muurolene	0.9
10	*β*-selinene	11.4
11	*α*-selinene	12.1
12	*δ*-cadinene	2.1
13	*γ*-cadinene	1.4
14	7-epi-*α*-selinene	5.7
15	Nonadecane	0.8
16	Eicosane	2.7
17	Caryophyllene oxide	2.1
18	Heneicosane	5.0
19	Hexahydrofarnesyl acetone	1.1
20	Docosane	4.7
21	Carvacrol	1.9
22	*α*-cadinol	0.7
23	Tricosane	3.3
24	Tetracosane	2.5
25	Pentacosane	1.0
26	Unidentified compound-1[#]	1.7
27	Unidentified compound -2[#]	0.6
28	Unidentified compound -3[#]	0.6
29	Unidentified compound -4[#]	0.7
30	Unidentified compound -5[#]	1.7
31	Unidentified compound -6[#]	1.4
32	Unidentified compound -7[#]	1.2
	Total	82.7

The phytochemical contents of botanical products vary significantly due to many factors, including genetics, climate, geography, and extraction methods, indicating the complex nature of botanical diversity and its implications for pharmacological discovery. This variability underscores the necessity of exploring plant chemovarieties to uncover unique compounds that may contribute to the rich tapestry of bioactive substances within natural product research.

MTT Assays

The ongoing searches for effective novel compounds to cure cancer highlights the significant role of natural products. Essential oils, with their antiproliferative effects, have recently gained attention and are among the natural products examined in the literature for their quality and efficacy. Studies have investigated the cytotoxic activities of extracts obtained from various plants of the *Stachys* species, including roots and aerial parts. A cytotoxic analysis study evaluated iridoids from 80% aqueous methanol extracts of *S. palustris* and *S. recta* using an *in vitro* MTT assay on three human tumor cell lines, with cisplatin and doxorubicin used as positive controls. Among the 30 samples examined, the root extract of *S. recta* was significantly active against breast carcinoma (MCF7 cell line) with >50% inhibition. Eight samples at a concentration of 10 µg/ml were moderately active (25–50% inhibition), with only *S. recta* inhibiting cell growth by more than 50%. In the A431 cell line, only the root extract of *S. recta* showed more than 25% inhibition, with an inhibition percentage of 41.84% at a concentration of 10 µg/ml. In the HeLa cell line, aucubin and harpagide exhibited more than 50% cytotoxic activity at 90 µg/ml [16]. Another study investigating cytotoxic activities of *S. laxa*, *S. trinervis*, *S. subaphylla*, and *S. turcomanica* collected from Iran worked on total methanol-water (80/20) extracts and fractions from the aerial parts of the plants. These fractions were tested for *in vitro* cytotoxic activity against colon carcinoma (HT-29), colorectal adenocarcinoma (Caco-2), breast ductal carcinoma (T47D), and Swiss mouse embryo fibroblast (NIH 3T3) cell lines using the MTT assay. Ethyl acetate and chloroform fractions of *S. laxa* and the chloroform and ethyl acetate fractions of *S. subaphylla* exhibited the highest cytotoxic activity against HT-29 and T47D cell proliferation (IC50 < 50 µg/ml) [17]. In another study, the methanolic extract, alkaloid, and terpenoid fractions of *S. pilifera* were evaluated for their *in vitro* (HT-29 cell line) cytotoxic and antiproliferative activities, yielding significant results. Cisplatin was used as a reference compound, and compared to other fractions the terpenoid fraction showed the best cytotoxic activity [10].

Antiproliferative Activity Analysis

The antiproliferative potential of SOBEO was assessed on the Caco-2 colon cancer cell line using the MTT assay. The assay results were evaluated at the 24, 48, and 72 h. after applying the studied sample to the cell line. Based on the MTT assay results, the highest effect was observed at the end of the 48th hour, where 5 µg of essential oil suppressed the growth of the Caco-2 cells by 41.56% (Figure 1, Table 2). This significant inhibition highlights the promising potential antiproliferative effect of the SOBEO, suggesting it could be a valuable natural source for the development of new agents against colon cancer.

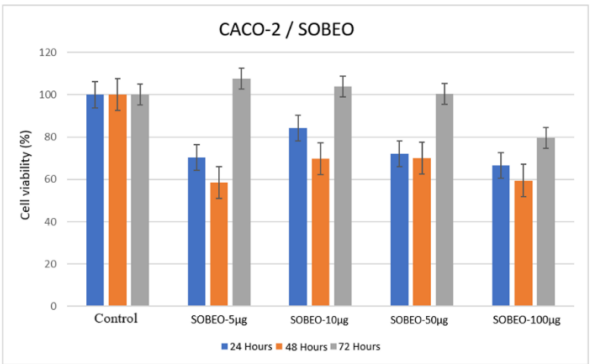

Figure 1. MTT analysis results of *Stachys officinalis* subsp. *balcanica* essential oil on Caco-2 cells after 24, 48 and 72 h

Table 2. Percent (%) change of *Stachys officinalis* subsp. *balcanica* essential oil in Caco-2 cell viability compared

Quantity	CACO-2 / SOBEO		
	24 h	*48 h*	*72 h*
5 µg	29.72 % ↓	41.56 % ↓	7.78 % ↑
10 µg	15.80 % ↓	30.12 % ↓	4.01 % ↑
50 µg	27.84 % ↓	30.01 % ↓	0.53 % ↑
100 µg	33.36 % ↓	40.49 % ↓	20.44 % ↓

↑ increase, ↓ decrease

Cytotoxic Activity Analysis

The cytotoxicity of SOBEO was assessed on the Vero cell line using the MTT assay method. After applying the studied sample to the Vero cells, the assay results were evaluated at the 24, 48, and 72 h. Based on the obtained results, no significant cytotoxic effect was observed on the Vero cells at any time points (24, 48, and 72 h) following the application of the samples (Figure 2, Table 3). This outcome indicates that while the essential oil exhibits potential antiproliferative effects against cancer cells, it does not adversely affect the viability of normal cell lines, highlighting its selectivity and suggesting a favorable safety profile for therapeutic applications.

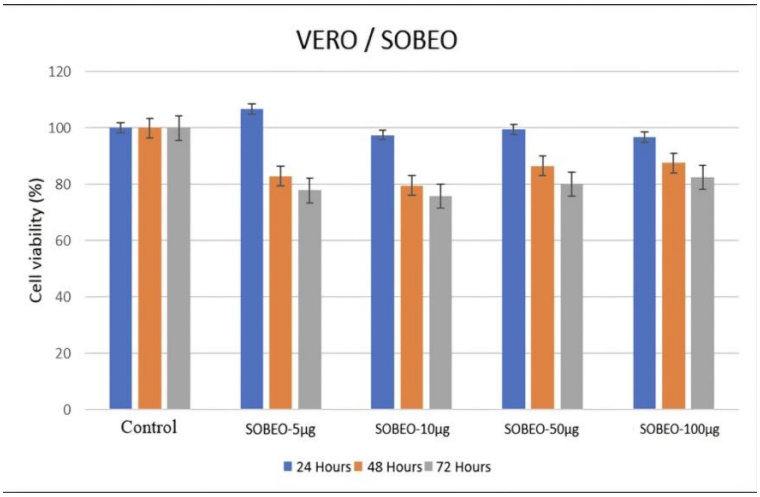

Figure 2. MTT analysis results of *Stachys officinalis* subsp. *balcanica* essential oil on Vero cells after 24, 48 and 72 h

Table 3. Percent (%) change of *Stachys officinalis* subsp. *balcanica* essential oil in Vero cell viability compared to control

Quantity	VERO / SOBEO		
	24 h	*48 h*	*72 h*
5 µg	6.71 % ↑	17.06 % ↓	22.09 % ↓
10 µg	2.53 % ↓	20.42 % ↓	24.12 % ↓
50 µg	0.49 % ↓	13.45 % ↓	19.86 % ↓
100 µg	3.24 % ↓	12.36 % ↓	17.41 % ↓

↑ increase, ↓ decrease

Conclusion

In this study the phytochemical content and potential anticancer effects of essential oil of *S. officinalis* subsp. *balcanica* were investigated. Both cytotoxic and antiproliferative activity analyses were conducted to assess its anticancer activities. A review of the current literature reveals that research on *S. officinalis* subsp. *balcanica* is sparse, with its phytochemical contents still needing to be fully elucidated. This investigation is the first study about the chemical components of *S. officinalis* subsp. *balcanica*, marking a significant contribution to the understanding of this taxon. Due to the lack of prior analyses on the cytotoxic and antiproliferative activities of *S. officinalis* subsp. *balcanica* essential oil, our findings indicate that its potential impact is slightly lower but still promising compared to other species within the *Stachys* genus. Applying GC/MS to SOBEO brought about the identification of unknown components. A thorough literature review based on the available mass spectra of these unidentified components could illuminate their chemical structures, addressing the knowledge gap. This pioneering report investigates the phytochemical profile and potential antiproliferative activity of *S. officinalis* subsp. *balcanica* and suggests that the essential oil of this plant could be a beneficial resource for medicinal applications. Furthermore, it underscores the potential for future research to enhance the economic significance of this plant.

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AUTHOR CONTRIBUTIONS

Concept: M.K., H.K.İ.Ö., D.C.D., B.E., H.G.S.İ.; Design: M.K., H.K.İ.Ö, D.C.D.; Control: H.K.İ.Ö., D.C.D., B.E., H.G.S.İ.; Sources: M.K., H.K.İ.Ö., D.C.D., B.E., H.G.S.İ.; Materials: H.K.İ.Ö., D.C.D.; Data Collection and/or Processing: M.K., H.K.İ.Ö., D.C.D., H.G.S.İ.; Analysis and/or Interpretation: D.C.D., B.E., H.G.S.İ.; Literature Review: M.K., H.K.İ.Ö., D.C.D.; Manuscript Writing: M.K., H.K.İ.Ö.; Critical Review: D.C.D., B.E., H.G.S.İ.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

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