DOI: 10.2478/fv-2026-0029

FOLIA VETERINARIA, 70, 3: 69—75, 2026

ORIGINAL ARTICLE

SELECTIVE INHIBITORY EFFECT OF CANNABIDIOL FROM *CANNABIS* SP. PLANTS ON TUMOUR CELL PROLIFERATION

Dana Marcinčáková[1]*, Mária Kolesárová[1], Natália Nosálová[2], Nikola Hudáková[3], Daša Čížková[2], Jaroslav Legáth[1]

[1]Department of Pharmacology and Toxicology, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia; [2]Small Animal Clinic, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia; [3]Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia

OPEN ACCESS

***Correspondence:** dana.marcincakova@uvlf.sk

Citation: Marcinčáková, D., Kolesárová, M., Nosálová, N., Hudáková, N., Čížková, D., Legáth, J., 2026: Selective inhibitory effect of cannabidiol from *Cannabis* sp. plants on tumour cell proliferation. Folia Veterinaria, 70, 3, 69–75.

Received: June 24, 2026

Accepted: August 31, 2026

Published: September 15, 2026

Copyright: 2026 Marcinčáková et al. This work is licensed under the Creative Commons Attribution-Non-Commercial-NoDerivatives 4.0 International License.

Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Cannabidiol is a non-psychoactive phytocannabinoid with growing interest as a potential antitumour agent in veterinary medicine due to its modulatory effects on cell proliferation and survival. The aim of this preliminary *in vitro* study was to evaluate the impact of cannabidiol on non-tumourigenic MCF-10A and tumourigenic MCF-7 breast cell lines using colorimetric measurement of metabolic activity (MTS assays) and the real-time cell analyser xCELLigence system (RTCA). Cannabidiol stimulated the metabolic activity of MCF-10A cells at all tested concentrations and time points, with maximal effects at 24 and 48 hours, while enhanced adhesion was observed after 72 hours. In contrast, MCF-7 cells showed a decrease in metabolic activity at 20 µM after 24 and 48 hours, alongside a consistent decrease in adhesion across all conditions, most pronounced at 48 hours. The Pearson correlation test revealed a strong positive correlation (r = 0.7768) between the MTS assay and xCELLigence system results. These findings suggest the selective effects of cannabidiol on tumour cells and support its potential as a targeted bioactive agent. To fully understand its efficacy in the context of various tumour types, more robust research into its mechanisms and safety is needed.

Keywords: adherence; breast cancer; MTS; proliferation; xCELLigence

INTRODUCTION

Cannabinoids are substances of the *Cannabis* sp. plant with a variety of pharmacological effects, including cannabidiol (CBD), a phytocannabinoid isolated from the *Cannabis sativa* plant, which exhibits a wide spectrum of biological effects. Unlike Δ9-tetrahydrocannabinol (THC), it does not have psychotropic properties. Its effect is mediated by interaction with the endocannabinoid system and other regulatory mechanisms of cells. CBD is used in both human and veterinary medicine and is characterized by anti-inflammatory, antioxidant, analgesic, and neuro-

protective effects [1]. Recently, attention has also been focused on the potential anti-tumour effects of CBD. Cannabinoids have been found to inhibit tumour proliferation, neovascularization, invasion, and chemoresistance [2, 3]. Many studies have shown that CBD exhibits high antitumour activity in breast cancers, reducing the proliferation of tumour cells [4, 5] and reducing breast cancer metastasis [6]. Some studies also report a positive effect of CBD in combination with conventional chemotherapy and radiotherapy, thereby improving antitumour efficacy [7, 8]. The mechanism of CBD's antiproliferative effect lies in its ability to induce apoptosis and influence cell migration and adhesion [9].

However, the specific molecular mechanisms underlying these multi-target effects remain unclear in the current literature. Furthermore, there is a lack of veterinary *in vitro* models for researching anti-cancer drugs for mammary gland tumours in animals; therefore, comparative veterinary oncology utilises well-characterized human cell lines. In this study, the MCF-7 cell line (a model of human epithelial breast adenocarcinoma expressing estrogen receptors) and the MCF-10A cell line (a non-tumourigenic human epithelial breast cell line serving as a standard for healthy tissue cells) were selected. Although these are human cell models, mammary gland tumours in companion animals (particularly canine mammary carcinomas) exhibit clinical, histological, and molecular-biological similarities to human breast cancer. Direct extrapolation of data from *in vitro* studies to animal systems has its limitations, primarily due to potential interspecies differences; nevertheless, this comparative approach serves to suggest future possibilities for in vivo studies.

Given the increasing incidence of mammary gland tumours in companion animals, the high toxicity of commercial chemotherapeutics, and the growing chemoresistance of tumours to them, investigating the mechanisms of the antiproliferative effect of CBD is necessary. In addition to the previously described effects in adjuvant therapy for pain relief and its direct antiproliferative effect against tumours, CBD may also potentially enhance the efficacy of conventional chemotherapeutic agents when used in combination, thereby helping to reduce the development of chemoresistance [10]. The aim of our preliminary experiment was to investigate the potential selective effect of CBD on tumour cells by monitoring the cellular response in real time. At the same time, the effect of CBD on the metabolic activity of tumour and non-tumour epithelial cell lines was evaluated.

MATERIALS AND METHODS

Preparation of the tested substance

A standard solution of cannabidiol – 1 mg/ml in methanol, certified reference material, 1 ml ampoule, Cerilliant®; CAS number: 13956-29-1 (Sigma-Aldrich, Germany) was used. The tested concentrations (2.5, 5, 10, and 20 µM) were selected based on relevant published literature mapping the in vitro therapeutic range of phytocannabinoids and our preliminary screening, ensuring clinical relevance without causing immediate non-specific solvent toxicity [2, 4]. The maximum concentration of methanol in the final culture medium did not exceed 0.5% (v/v), and an equivalent vehicle control (solvent control containing 0.5% methanol) was included in all assays to rule out vehicle-induced cytotoxicity.

Cell cultivation

Experiments were performed using the human breast cancer cell line MCF-7 and the non-cancerous epithelial breast cell line MCF-10A (American Type Culture Collection (ATCC, Manassas, VA, USA)). MCF-7 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Biosera, Kansas City, MO, USA) supplemented with 2% antibiotic–antimycotic solution (ATB/ATM; penicillin (100 UI/mL), streptomycin (100 µg/mL), amphotericin B (2.5 µg/mL), Lonza, Switzerland), 10% foetal bovine serum (FBS), 1% L-glutamine, and 0.1% gentamicin (Sigma, United States). A high-glucose DMEM/F12 growth medium (Biosera, Kansas City, MO, USA) supplemented with 10% FBS, 2% ATB/ATM solution, insulin (10 µg/mL), epidermal growth factor (EGF; 20 ng/mL), and hydrocortisone (0.5 µg/mL) was used for the cultivation of the MCF-10A cell line. Cells were maintained in an incubator under standard culture conditions in a humidified atmosphere containing 5% CO_2 at 37 °C. The culture medium was replaced every 48 hours with freshly prepared medium, and cells were monitored using an inverted microscope (Zeiss Axiovert 200) equipped with a digital camera. When cell confluence reached approximately 80 – 90%, cells were passaged by enzymatic dissociation using trypsin (Sigma, United States). Cell viability exceeded 95% prior to each experiment.

Evaluation of metabolic activity changes using the MTS assay

The MTS assay (CellTiter 96 non-radioactive cell proliferation assay, Promega, Madison, WI, USA) quantifies the metabolic activity (MA) of cells *in vitro* based on their viability in a cytotoxic assay. It relies on the reduction of a tetrazolium component (MTS) by viable cells into a coloured, soluble formazan product that absorbs light. The intensity of this colour is directly proportional to the number of living cells and measured spectrophotometrically. In this study, both cell lines were plated in three 96-well plates (3,000 cells per well in 100 µl growth medium). Cells were treated with the test substance at the selected concentrations in a 1:10 ratio to the medium. The MTS assay was performed after 24, 48, and 72 hours of incubation according to the manufacturer's instructions. The plates were then incubated for an additional 4 hours. The amount of formazan formed was evaluated spectrophotometrically using an LB 913 Apollo 11 ELISA reader (Berthold Technologies, Bad Wildbad, Germany) at a wavelength of 450 nm. The absorbance obtained was compared with the control (100%) and expressed as a percentage according to the formula: *% metabolic activity = (Sample absorbance) x 100 / (Control absorbance)*.

Real-time cell monitoring and EC50 determination using the xCELLigence system

The xCELLigence system, a real-time cell analyser (RTCA) dual-plate instrument (ACEA Bioscience Inc., San Diego, CA, USA), allows continuous monitoring of cell adhesion properties *in vitro* in a non-invasive and label-free manner. Two 16-well E-plates (ACEA Bioscience Inc., San Diego, CA, USA) were used in the experiment – one for each cell line. First, 100 µl of growth medium was added to the wells for background measurement. Then, a suspension containing 3,000 cells (in 80 µl of medium) was added. The plates were placed back into the incubator and incubated for 24 h to allow the proliferating cells to form a 90% monolayer. After 24 h, selected concentrations of CBD (2.5 – 20 µM) were added to the cells at a ratio of 1:10 with the medium. Any changes in adhesion generate a value known as electrical impedance, which is measured throughout the experiment (72 h). These values are converted by the software into dimensionless values known as Cell Index (CI) and recorded by the xCELLigence software as a graph and displayed as "Normalised Cell Index" (CI = 1) versus time. The CI values were used to calculate the proliferative activity (PA), expressed as a percentage according to the formula: *Proliferative activity % = CI sample x 100 / CI control*. All calculations were performed based on a minimum of three independent biological replicates, with each condition tested in triplicates.

Statistical evaluation

The acquired data were statistically analysed using GraphPad Prism version 10.3.1 (GraphPad Software, San Diego, CA, USA). The values were expressed as percentages of metabolic activity (MTS) and cell adherence (RTCA), with control cells (unaffected) set at 100%. The values were compared to the control using the ANOVA multiple comparisons test with Dunnett's corrections; we also compared the results at 24, 48, and 72-hour intervals at different concentrations between the MCF-10A (non-tumour) and MCF-7 (tumour) cell lines using multiple t-tests. The significance level was set at $p < 0.05$. We determined the relationship between MTS and RTCA values using Pearson's correlation test, where coefficients less than 0.3 represent a weak linear correlation, 0.3 – 0.5 a moderate one, and greater than 0.5 a strong linear correlation.

RESULTS

The results of both our analyses (MTS, RTCA) of the cells demonstrated that cannabinoids have a stimulating effect on both the adhesion and metabolic activity of non-tumour MCF-10A cells compared to control cells not exposed to CBD. Regarding metabolic activity, this was observed at all tested cannabinoid concentrations across all time intervals (Figure 1), with the highest values observed after 24 and 48 hours. In the case of adhesion, we observed stimulation only after 72 hours of CBD exposure (Figure 2). In contrast, however, we did not observe any metabolic activity or adhesion-promoting effects in the MCF-7 tumour cell model. At a concentration of 20 µM, we observed significantly reduced metabolic activity after 24 and 48 hours compared to the control (without CBD). Regarding adhesion, we observed a significant decrease in adhesion compared to the control group at all concentrations tested and at all time points, with the most pronounced decrease occurring after 48 hours of CBD exposure. The Pearson correlation coefficient $r = 0.7768$ confirmed a highly significant, strong positive linear correla-

tion ($P < 0.001$) between MTS and RTCA results (Table 1).

We confirmed the selectivity of CBD toward tumour cells using paired t-tests, in which metabolic activity (MTS) and adhesion (RTCA) were compared between MCF-10A and MCF-7 cells at the same time points and concentrations (Table 1). The results confirmed significant differences in metabolic activity and adhesion between non-tumor and tumor cells at all observed CBD concentrations for metabolic activity after 48 and 72 hours of cultivation. Similarly, we observed significant differences in adhesion at all concentrations tested after 48 and 72 hours of cultivation, with the greatest difference observed after 72 hours at 5 µM CBD.

DISCUSSION

Interest in the study and use of phytocannabinoids has been growing, and their potential use in various diseases is currently being explored. Regarding cancer, cannabinoids are primarily used to alleviate the side effects of chemotherapy, but a growing number of studies now attribute antiproliferative, proapoptotic, anti-invasive, and antiangiogenic effects to these compounds [11, 12, 13].

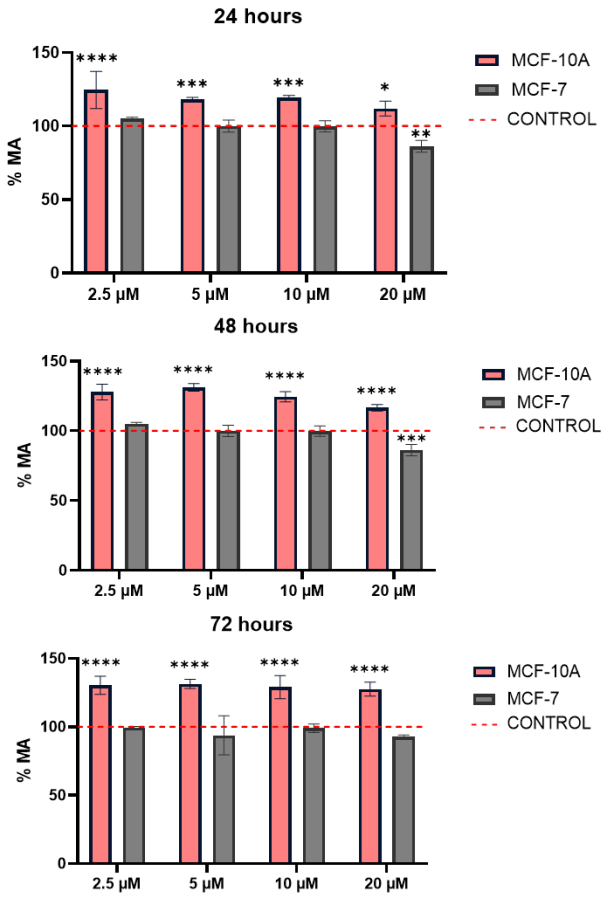


Fig. 1. The effect of CBD on the metabolic activity of MCF-10A and MCF-7 cell lines after 24, 48 and 72 hours of incubation. The graph represents the percentage of metabolic activity (MA) as measured by the MTS test in comparison with control cells (no agent added). ANOVA * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001 statistically different to control cells.

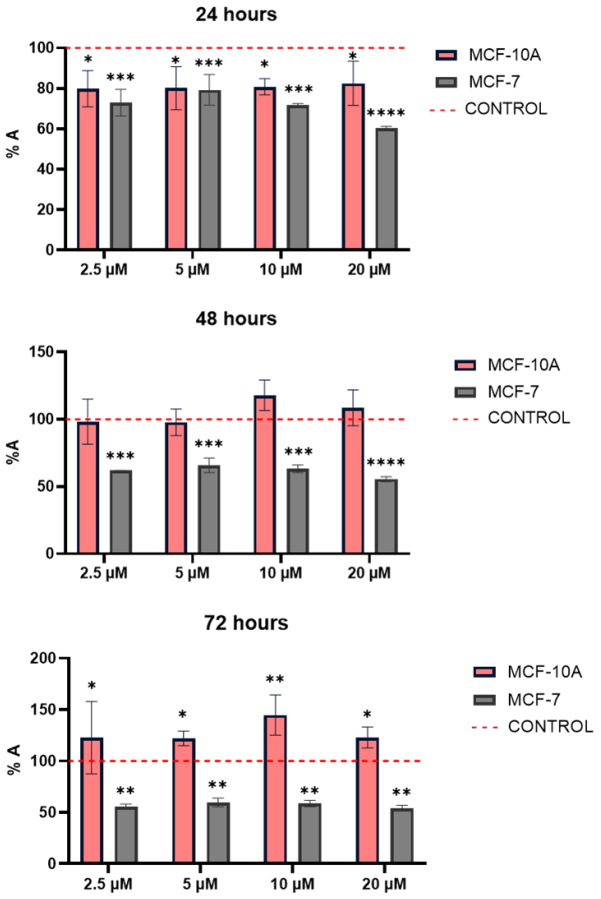


Fig. 2. The effect of CBD on cell adherence of MCF-10A and MCF-7 cell lines after 24, 48 and 72 hours of incubation. The graph represents the average percentage of cell adherence (A) as measured by the RTCA test in comparison with control cells (no agent added). % A – percentage of adherence, ANOVA * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001 statistically different to control cells.

The results of our study demonstrated a selective antiproliferative effect of CBD in a concentration-dependent manner on the MCF-7 mammary tumour cell line compared to the control. In the case of the non-tumourous breast epithelial cell line MCF-10A, CBD showed a concentration-dependent effect on cell proliferation compared to the control. These results were confirmed by MTS and RTCA methods. The most significant changes in cell proliferation inhibition were observed in the MCF-7 tumour cell line and in proliferation promotion in the MCF-10A cell line after 72 hours. A similar antiproliferative effect was also noted in a study [2], which demonstrated inhi-

Table 1. Pairwise comparations (t-test)

Variables (MCF-10A vs. MCF-7)	MTS			RTCA		
	24 h	48 h	72 h	24 h	48 h	72 h
CBD 2.5 µM	0.055387	0.002264	0.001312	0.343158	0.01991	0.029784
CBD 5 µM	0.001904	0.000398	0.011385	0.919528	0.00794	0.000201
CBD 10 µM	0.001057	0.00127	0.004551	0.018149	0.001254	0.00165
CBD 20 µM	0.002326	0.000359	0.000326	0.024255	0.002406	0.00036

* The table shows the corresponding p-values from the multiple t-tests comparing the percentage of metabolic activity (for MTS) and the mean percentage CI (for RTCA) between MCF-10A (non-tumour) and MCF-7 (tumour) cells at 24, 48, and 72 h. Multiple T-tests, statistical significance $P < 0.05$.

bition of proliferation of both MDA-MB-231 and MCF-7 breast tumour cell lines, with an IC50 value of 11.37 ± 1.82 µM and 20.04 ± 2.97 µM after 48 hours of CBD incubation. The cytotoxic effects of CBD in a range of concentrations (0–15 µM) were recorded after 24, 48 and 72 hours in the Head and neck squamous cell carcinoma (HNSCC) tumour cell line, which includes four tumour cell lines (FaDu human hypopharyngeal squamous cell carcinoma line, SNU899 human larynx squamous cell carcinoma line, SCC15 human tongue squamous cell carcinoma line, and Hep2 human epidermoid laryngeal carcinoma line). The greatest decrease in viability was recorded after 48 – 72 hours after treatment of cells with CBD (< 6 µM) compared to the control normal human oral keratinocyte cell line (HOK), indicating an antitumour effect of CBD. At the same time, the antimigratory and anti-invasion effect of CBD on human HNSCC cell lines was recorded [14].

Cannabinoids confer anti-tumour activity to them by inducing apoptosis through cannabinoid receptor 1 and 2-dependent stimulation. Although this mechanism is less clear for CBD, studies suggest that CBD may promote apoptotic death of cancer cells independently of CB1 and CB2 receptors. Regardless of the involvement of cannabinoid receptors, the promotion of apoptotic cell populations in cancer cells appears to be due to the ability of cannabinoids to stimulate ROS production. Furthermore, studies have shown that CBD can trigger glutathione (GSH) depletion, which exacerbates oxidative stress and cell death in human glioma cells. Since these specific downstream molecular pathways (ROS accumulation and GSH depletion) were not experimentally verified in our current study, it is crucial to emphasize that while these mechanisms are widely described in other cancer models [15], this premise remains a theoretical assumption that must be validated for our specific lines in future research.

An essential factor is the selective effect of CBD on tumour and healthy tissues. Comparable to our findings, several preclinical studies have shown that CBD concentrations cytotoxic to cancer cell lines did not significantly reduce the viability of healthy cells, such as primary glial cultures (up to 50 µM CBD), a human oral keratinocyte cell line (up to 15 µM for 24 hours), rat preadipocytes, and mouse monocyte and macrophage cell lines (10 µM for 72 hours) [15]. CBD should therefore be considered a multi-targeted bioactive compound capable of eliciting various responses depending on the cellular microenvironment, which includes the specific receptor profile, CBD concentration, and bioavailability [15].

There are several critical limitations in this preliminary study. First, we evaluated the response of only one non-tumourous and one tumourigenic cell line, and second, a direct veterinary cell line model was absent. This limits immediate species-specific conclusions. Although research on cannabinoids, particularly products containing CBD, has a long history in human medicine, this field is still in its early stages in veterinary medicine. Future studies should therefore employ animal models to explore potential applications of cannabinoids in adjuvant therapy for companion animals.

CONCLUSION

Cannabinoids have recently gained scientific interest due to their wide range of pharmacological effects. In our experiment, CBD demonstrated increased metabolic activity and adhesion in non-tumour MCF-10A cells, while inhibiting these parameters in tumour MCF-7 cells. These findings suggest that CBD can selectively influence cell behaviour, promoting the activity of non-tumour cells while suppressing the activity of tumour cells

and support the hypothesis that CBD is a promising agent in anti-cancer therapy with a selective effect. While these preliminary in vitro results provide an important baseline for comparative oncology, they do not support immediate or direct therapeutic application in clinical veterinary practice. In the context of veterinary pharmacology, future systematic in vitro studies utilizing specific veterinary cell lines, followed by robust in vivo animal trials, are strictly required to thoroughly elucidate the precise molecular pathways and establish clinical safety and pharmacokinetic efficacy.

Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

Ethical Statement

Ethical approval was not necessary for this work.

Conflict of Interest

No conflict of interest.

Funding

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-24-0026, by the Science Grant Agency – project VEGA 1/0236/23 and by National Reference laboratory for pesticides University of Veterinary Medicine and Pharmacy in Košice.

Generative AI Statement

No generative artificial intelligence (AI) and AI-assisted technologies were used in writing the manuscript.

Authors' Contributions

DM designed the study and analysed the data; NN carried out the study; NH collected the data; MK and NH provided literature search and resources; DČ and JL proofread and approved the manuscript.

REFERENCES

1. **Ibeas Bih, C., Chen, T., Nunn, A. V. W., Bazelot, M., Dallas, M., Whalley, B. J., 2015:** Molecular targets of cannabidiol in neurological disorders. *Neurotherapeutics*, 12, 4, 699–730. DOI: 10.1007/s13311-015-0377-3
2. **Fraguas-Sánchez, A. I., Martín-Sabroso, C., Torres-Suárez, A. I., 2018:** Insights into the effects of the endocannabinoid system in cancer: A review. *Br. J. Pharmacol.*, 175, 13, 2566–2580. DOI: 10.1111/bph.14331
3. **Hinz, B., Ramer, R., 2019:** Anti-tumour actions of cannabinoids. *Br. J. Pharmacol.*, 176, 10, 1384–1394. DOI: 10.1111/bph.14426
4. **Ligresti, A., Moriello, A. S., Starowicz, K., Matias, I., Pisanti, S., De Petrocellis, L., et al., 2006:** Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol on human breast carcinoma. *J. Pharmacol. Exp. Ther.*, 318, 3, 1375–1387. DOI: 10.1124/jpet.106.105247
5. **Sultan, A. S., Marie, M. A., Sheweita, S. A., 2018:** Novel mechanism of cannabidiol-induced apoptosis in breast cancer cell lines. *Breast*, 41, 34–41. DOI: 10.1016/j.breast.2018.06.009
6. **McAllister, S. D., Murase, R., Christian, R. T., Lau, D., Zielinski, A. J., Allison, J., et al., 2011:** Pathways mediating the effects of cannabidiol on the reduction of breast cancer cell proliferation, invasion, and metastasis. *Breast Cancer Res. Treat.*, 129, 1, 37–47. DOI: 10.1007/s10549-010-1177-4
7. **Scott, K. A., Dalgleish, A. G., Liu, W. M., 2017:** Anticancer effects of phytocannabinoids used with chemotherapy in leukaemia cells can be improved by altering the sequence of their administration. *Int. J. Oncol.*, 51, 1, 369–377. DOI: 10.3892/ijo.2017.4022
8. **Çifçi, K. U., Çelik, A. B., Güçlü, E., Şahinoğlu, N., Gülüm, L., Çapkınoğlu, E., Tutar, Y: 2026:** The effect of cannabidiol on cancer-pathway genes in doxorubicin-sensitive and resistant breast cancer cells. *Pharmaceuticals*, 19, 4, 615. DOI: 10.3390/ph19040615
9. **Almeida, C. F., Correia-da-Silva, G., Ribeiro, A. P., Teixeira, N., Amaral, C., 2025:** Impact of minor cannabinoids on key pharmacological targets of estrogen receptor-positive breast cancer. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids*, 1870, 6, 159658. DOI: 10.1016/j.bbalip.2025.159658
10. **Almeida, C. F., Teixeira, N., Correia-da-Silva, G., Amaral, C., 2022:** Cannabinoids in breast cancer: Differential susceptibility according to subtype. *Molecules*, 27, 1, 156. DOI: 10.3390/molecules27010156

11. **Ma, L., Liu, M., Liu, C., Zhang, H., Yang, S., An, J., et al., 2024:** Research progress on the mechanism of the antitumor effects of cannabidiol. *Molecules*, 29, 9, 1943. DOI: 10.3390/molecules29091943
12. **Klumpers, L. E., Thacker, D. L., 2019:** A brief background on cannabis: From plant to medical indications. *J. AOAC Int.*, 102, 2, 412–420. DOI: 10.5740/jaoacint.18-0208
13. **Alves, P., Amaral, C., Teixeira, N., Correia-da-Silva, G., 2020:** *Cannabis sativa*: Much more beyond Δ9-tetrahydrocannabinol. *Pharmacol. Res.*, 157, 104822. DOI: 10.1016/j.phrs.2020.104822
14. **Śledziński, P., Zeyland, J., Słomski, R., Nowak, A., 2018:** The current state and future perspectives of cannabinoids in cancer biology. *Cancer Med.*, 7, 3, 765–775. DOI: 10.1002/cam4.1312
15. **Go, Y. Y., Kim, S. R., Kim, D. Y., Chae, S.-W., Song, J.-J., 2020:** Cannabidiol enhances cytotoxicity of anti-cancer drugs in human head and neck squamous cell carcinoma. *Sci. Rep.*, 10, 1, 20622. DOI: 10.1038/s41598-020-77674-y