Clinical and Experimental Health Sciences

A Preliminary Investigation of *Stachys subnuda's* Anticancer Effects on the MCF-7 Breast Cancer Cell Line

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ABSTRACT

Objective: *Stachys subnuda*, traditionally used in Anatolia for various ailments, is a medicinal plant with known therapeutic potential. Previous studies have already demonstrated the antioxidant and anti-inflammatory properties of Stachys species. Since the major secondary metabolites of Stachys subnuda are known to possess cytotoxic activity against breast cancer, the aim of this study is to evaluate the potential anticancer effects of *Stachys subnuda* methanol extract and its n-hexane, chloroform, ethyl acetate, and aqueous methanol fractions.

Methods: Maceration and liquid-liquid extraction methods were used to obtain *Stachys subnuda* methanol extract and its fractions. The CCK-8 test was applied to MCF-7 breast cancer cells to calculate IC_{50} values. The depolarization of mitochondrial membrane potential was examined using the JC-1 assay. Bax/Bcl-2 and caspase-7 protein levels were determined by western blot.

Results: The n-hexane fraction exhibited significant cytotoxicity, reduced cell viability, depolarized mitochondrial membrane potential, and induced apoptosis through mitochondrial pathways. This was further supported by an increase in the Bax/Bcl-2 ratio and elevated caspase-7 protein level.

Conclusion: The n-hexane fraction of *Stachys subnuda* holds promise as a potential therapeutic agent for breast cancer by targeting mitochondrial-mediated apoptotic pathways. Further studies are warranted to elucidate the active compounds and their mechanisms of action.

Keywords: *Stachys subnuda*, Lamiaceae, breast cancer, MCF-7, apoptosis

1. INTRODUCTION

Cancer is a multifactorial disease characterized by uncontrolled and abnormal cell proliferation (1). In the United States alone, more than 2 million new cancer cases are projected by 2025 (2). Breast cancer ranks as the most prevalent type of cancer in women (3). The survival rate of breast cancer has increased thanks to effective treatments such as chemotherapy. However, it has been observed that chemotherapy has long-term disadvantages, including neuropathy and heart failure, and negatively impacts quality of life (4, 5).

Scientific exploration of plant-derived anticancer agents dates back to the 1950s. In recent decades, the use of natural compounds in cancer therapy has gained increasing attention due to their diverse bioactivities and relatively lower toxicity profiles. Phytochemicals such as phenols, flavonoids,

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terpenoids, and alkaloids play pivotal roles in this context. Members of the Lamiaceae family, in particular, have been traditionally utilized for cancer prevention and treatment. Among their abundant secondary metabolites, polyphenols and terpenes are the most prominent, and these compounds are believed to exert anticancer effects primarily by inducing apoptosis in malignant cells (6, 7).

The genus *Stachys* is one of the largest within the *Lamiaceae* family and encompasses numerous species with a long history of use in traditional medicine across various cultures (8). *Stachys subnuda,* an endemic species growing wild in the eastern Anatolia region of Turkey, is commonly known as "mountain tea" (9, 10). The synergistic effects of the compounds may explain why flowers and aerial parts are consumed as tea (11).

In a previous study, the antioxidant and anti-inflammatory effects of the essential oil obtained from the aerial parts of *Stachys subnuda* were investigated *in vitro*. Additionally, the chemical composition of *Stachys subnuda* essential oil was also demonstrated. The most abundant compounds in *Stachys subnuda* essential oil were determined as phytol (25.2%) and β-caryophyllene (11.2%) (12). Phytol has been reported to exert cytotoxic effects on MCF-7 breast cancer cells (13), and its genotoxic and apoptotic properties have also been confirmed *in vivo* (14). Likewise, β-caryophyllene has been shown to significantly inhibit the proliferation of MDA-MB-468 triple-negative breast cancer cells (15). Two acylated flavonoids derived from an ethyl acetate extract fraction of the aerial part of *Stachys subnuda* were also shown to have anti-inflammatory and antioxidant properties (16).

Although there is no biological and chemical study on *Stachys subnuda* species, studies on other *Stachys* species have revealed that secondary metabolites are generally iridoid and flavone glycosides, terpenes (sesquiterpenes, diterpenes) (12, 17). Various iridoid glycosides have been shown to inhibit cell survival and proliferation and induce apoptosis against breast cancer. Their anti-tumor properties have also been determined *in vivo* (18).

In this study, the cytotoxic and apoptotic effects of *Stachys subnuda* methanol extract (SSM) and its hexane (SSH), chloroform (SSC), ethyl acetate (SSEA), and aqueous methanol (SSAM) fractions on breast cancer were investigated for the first time by *in vitro* methods. The selection of this specific plant was fundamentally guided by its strong ethnobotanical background in Anatolia and its promising phytochemical profile rich in cytotoxic terpenes.

2. METHODS

2.1. Plant Material and Extraction

The above-ground parts of *Stachys subnuda* were collected during the flowering period from Tunceli province of Turkey. It was diagnosed by Dr. Ahmet Doğan. A few specimens of the plant are registered in Marmara University Faculty of Pharmacy Herbarium (MARE) with number 17720. Dried and ground aerial parts of *Stachys subnuda* (10 g) were macerated with methanol (3x100 mL) using an ultrasonic bath. After the filtration process, the solvent of the methanol extract obtained was evaporated in a rotary evaporator, and the methanol extract in dry powder form was obtained (SSM). After the liquid-liquid extraction method, the solvents of *n*-hexane, chloroform, ethyl acetate, and aqueous methanol fractions were evaporated in a rotary evaporator, and hexane (SSH), chloroform (SSC), ethyl acetate (SSEA), and aqueous methanol (SSAM) fractions were obtained, respectively.

2.2. Cell Culture

MCF-7 cells (ATCC HTB-22) were cultured at 37°C in a carbon dioxide incubator with 5% CO_2 in DMEM culture medium with 10% FBS, 100 U/ml penicillin, and 0.1 mg/ml streptomycin. The media was changed every two days. Trypan blue staining was used to evaluate cell viability (19).

2.3. Cell Viability and Cytotoxicity

MCF-7 cells were exposed to several doses of *Stachys subnuda* extract/fractions (10–100 µg/mL) for 24 hours in order to assess cytotoxicity and half-maximal inhibitory concentration (IC_{50}) values. The procedure used by the manufacturer was followed (Cell Counting Kit-8, KTC011001, Abbkine). The experiment was performed in three replicates (n=3). Cell Counting Kit-8 (CCK-8) is designed to detect cell proliferation, while cell toxicity based on WST-8 is reduced by dehydrogenases in cells to yield an orange-colored product (formazan).

2.4. Mitochondrial Membrane Potential Assay

After the cells were seeded at $5x10^4$, incubations were made with SSH in different concentration ranges (5, 10, 15, 20, 25 µg/mL). After 24 hours, JC-1 was applied according to the manufacturer's protocol (JC-1 Mitochondrial Membrane Potential Assay Kit, 10009172, Cayman Chemical). The experiment was performed in three replicates (n=3). While JC-1 enters the mitochondria and gives red fluorescence in healthy cells with high mitochondrial potential, it remains in monomeric form and gives green fluorescence in cells with low potential that have undergone apoptosis. Using a microplate reader (Synergy H1, BioTek Instruments Inc., Vermont, USA), the ratio of apoptotic to healthy cells was measured by reading in two distinct fluorescence channels (535/595 for healthy cells, 485/535 for apoptosis).

2.5. Western Blot

Following a 24-hour incubation period with different concentrations of SSH, the methods utilized for membrane transfer, electrophoresis, and cell lysis were the same as in our earlier study (20). The experiment was performed in two replicates (n=2). Primary antibodies used were β-actin (Novus Biologicals, NB600-501), Bax (Novus Biologicals, NBP2-32809),

Bcl-2 (Novus Biologicals, NBP2-15200), and caspase-7 (Novus Biologicals, NBP3-14866). HRP chemiluminescent substrates (WesternBright ECL HRP Substrate, K-12045-050, Advansta) were used to identify the bands. Protein bands were imaged (Celvin S, Biostep) and quantified (ImageJ, version 1.54).

2.6. Statistics

The obtained data was statistically analyzed using GraphPad Prism software (version 8.0.1, GraphPad Software, USA). Tukey's multiple comparison test was conducted after the one-way ANOVA test. Statistical significance was defined as *P<.05 and **P<.01.

3. RESULTS

3.1. Cell Viability and Cytotoxicity

The IC_{50} values calculated after the 24-hour effect of *Stachys subnuda* extract/fractions on MCF-7 cells are shown in Table 1. The lowest IC_{50} value was obtained for SSH with 16.44 ± 0.96 µg/mL. This shows that SSH exhibits higher cytotoxicity and provides more effective inhibition on MCF-7 cells compared to other extract/fractions tested. In contrast, the IC_{50} value of SSM was measured as 30.45 ± 0.09 µg/mL and that of SSAM was measured as 34.18 ± 0.12 µg/mL. These values are approximately two times higher than those of SSH, indicating that these samples have lower cytotoxic effects. It can be said that at the concentration where SSH is effective, SSM and SSAM limit cell proliferation less effectively. SSC and SSEA fractions have IC_{50} values of 77.73 ± 0.09 µg/mL and 70.07 ± 0.03 µg/mL, respectively. These high IC_{50} values suggest that these fractions did not show significant cytotoxic effects on the tested cell line and required much higher concentrations to suppress cell growth. This implies that, in comparison to SSH, SSC and SSEA showed less biological activity and had little therapeutic promise. Due to these factors, only SSH was utilized for additional analysis.

Table 1. *CCK-8 test results in MCF-7 cells for 24h (given as mean ± SD).*

Stachys subnuda extract/fractions	IC_{50} (µg/mL for 24h)
SSM	30.45 ± 0.09
SSH	16.44 ± 0.96
SSC	77.73 ± 0.09
SSEA	70.07 ± 0.03
SSAM	34.18 ± 0.12

Stachys subnuda methanol extract and its n-hexane, chloroform, ethyl acetate and aqueous methanol fractions are coded as SSM, SSH, SSC, SSEA and SSAM, respectively.

When the absorbance graph for SSH (Figure 1) was examined, it was determined that the absorbance value decreased significantly at 25 µg/mL and above.

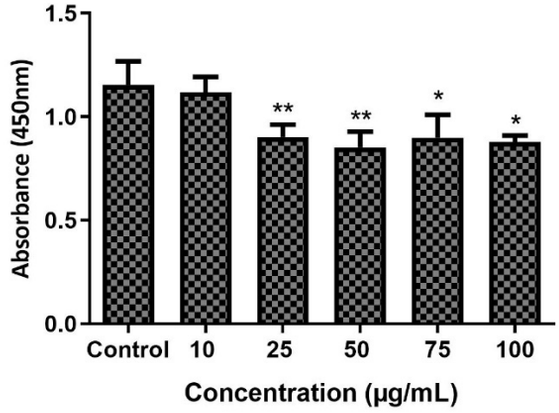


Figure 1. *Absorbance values of SSH for 24h on MCF-7 cells. (*P<.05; **P<.01 compared to the control group)*

3.2. Effect of SSH on Mitochondrial Membrane Potential

According to Wang and Youle (2009), mitochondria are necessary for triggering apoptosis in mammalian cells (21). Numerous natural substances work by interfering with mitochondrial processes. Ca2+ ions are released from the endoplasmic reticulum, mitochondrial permeability transition pores open, cytochrome-c is released, caspases are activated, and mitochondria rapidly depolarize during this process (22).

In our study, the depolarization of mitochondrial membrane potential observed when increasing concentrations of SSH (5, 10, 15, 20, 25 µg/mL) are applied is shown in Figure 2. Depolarization of the mitochondrial membrane potential increased at a statistically significant level in comparison to the control group at SSH dosages of 15 µg/mL and higher. This suggests that SSH targets mitochondrial functions through direct or indirect effects on mitochondria.

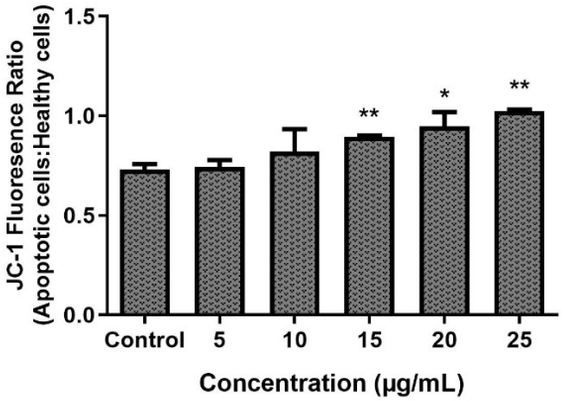


Figure 2. *Mitochondrial membrane potential depolarization effects of SSH for 24 h on MCF-7 cells. (*P<.05; **P<.01 compared to the control group)*

3.3. Effect of SSH on Apoptotic Proteins

In this study, the Bax/Bcl-2 ratio and caspase-7 protein level were examined by the Western blot method after SSH treatment at different concentrations, and the results are shown in Figure 3.

Our findings showed that the Bax/Bcl-2 ratio and caspase-7 protein level were statistically significantly higher than those of the control group at SSH dosages of 50 µg/mL and above.

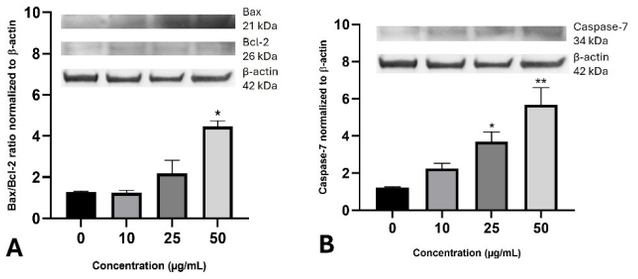


Figure 3. *Expression of proteins and graphical quantification for (A) Bax/Bcl-2 ratio and (B) caspase-7 normalized to β-actin after 24h incubation. (*P<.05; **P<.01 compared to the control group)*

4. DISCUSSION

Determining IC_{50} values on cancer cell lines is one of the most widely applied *in vitro* approaches for assessing cytotoxic potential (23). A medicinal product with a low IC_{50} value is effective at low doses and thus causes less systemic toxicity when given to the patient (24). Among the tested extracts, SSH yielded the lowest IC_{50} value at 24 hours and demonstrated dose-dependent cytotoxicity. According to the American National Cancer Institute (NCI), a crude herbal extract is generally considered to have potent anticancer activity when IC_{50} < 20 µg/mL (25). Based on this criterion, SSH, with an IC_{50} value of 16.44 ± 0.96 µg/mL, showed a strong cytotoxic effect.

Since no cytotoxicity studies have previously been reported for *Stachys subnuda* on breast cancer, our findings can only be compared with those of other *Stachys* species. In earlier reports, the IC_{50} value in MCF-7 cells was calculated for the hydroalcoholic extract of *Stachys setifera* as 827.52 µg/mL (26), for the dichloromethane extract of *Stachys circinata* as 3.67 mg/mL (27), and for the hydroalcoholic extract of *Stachys inflata* as 396 ± 6.9 µg/mL (28). Compared with these values, SSH markedly suppressed MCF-7 cell viability at significantly lower concentrations.

Our previous phytochemical analyses revealed that *Stachys subnuda* contains phenolic acids (quinic acid, 5-caffeoylquinic acid), flavonoids (isoscutellarein derivatives), and terpenes (phytol, β-caryophyllene, germacrene D). In addition, the hexane fraction was shown, both qualitatively and quantitatively, to contain phenolic compounds (phenolic acids, hydroxylated flavonoids) and flavonoids using the Folin–Ciocalteu and AlCl3 methods (12, 16). Phenolic acids, flavonoids, and terpenes are widely recognized for their anticancer potential, particularly against breast cancer (29, 30). These compound classes may therefore underlie the cytotoxic and anti-proliferative effects of SSH in MCF-7 cells.

The therapeutic potential of plant extracts strongly depends on the solvent used for extraction. Consequently, it is important to evaluate a wide range of solvents (31). Polar (water, methanol), intermediate polar (ethyl acetate), and nonpolar (n-hexane, chloroform) solvents are all commonly used in medicinal plant research (32). In our study, *n*-hexane extract gave the most effective result in terms of cell viability. Although there are various studies on the toxicity of hexane, it is a solvent that is currently widely used in the food industry to extract various compounds (33, 34). A study investigating the cytotoxicity of volatile, water-insoluble organic solvents on hepatocytes demonstrated that *n*-hexane was the least cytotoxic among the tested solvents (35).

Proteins of the Bcl-2 family are critical modulators of apoptosis. Within this family, the pro-apoptotic protein Bax promotes cell death, while the pro-survival protein Bcl-2 exerts an opposing effect (36). Additionally, the completion of apoptosis depends on functioning caspases. Caspases 3, 6, and 7 are involved in the execution of apoptosis (37). MCF-7 cells are known to lack caspase-3; however, caspase-7 can function as an alternative executioner, mediating mitochondrial-dependent apoptosis (38, 39). In our study, an increased Bax/Bcl-2 ratio indicated activation of the mitochondrial pathway, while caspase-7 activation confirmed completion of the executioner phase of apoptosis. This conclusion was further supported by the observed depolarization of the mitochondrial membrane potential. This finding is highly consistent with similar studies in the literature, which report that extracts from the Stachys species exert their anticancer activity primarily by disrupting the mitochondrial membrane potential (40, 41) and shifting the balance of Bcl-2 family proteins towards apoptosis (42).

These results suggest that the dose-dependent effects of SSH occur through interconnected cellular mechanisms, specifically targeting the mitochondrial pathway to induce apoptosis. Such findings provide a valuable basis for future research aimed at evaluating the therapeutic potential of SSH.

Our study has several strengths that distinguish it from previous reports. This is the first study to demonstrate the cytotoxic and pro-apoptotic activity of *Stachys subnuda* on breast cancer cells. Unlike studies with other *Stachys* species, SSH exhibited a remarkably lower IC_{50} value, well below the NCI threshold for potent anticancer activity. Therefore, SSH exerts strong cytotoxic efficacy even at low concentrations. Importantly, beyond assessing cytotoxicity, we also elucidated the underlying mechanisms, linking Bax/Bcl-2 modulation, caspase-7 activation, and mitochondrial membrane depolarization to the observed effects. In this way, we provided mechanistic insights that were not addressed in previous reports. Moreover, the detailed phytochemical background of S. subnuda obtained from our earlier studies

strengthens the biological plausibility of our findings by correlating specific compound classes with the observed anticancer effects. Together, these features highlight the novelty and robustness of our work, supporting SSH as a promising candidate for further preclinical investigations.

5. CONCLUSION

This study demonstrated the cytotoxic and apoptotic effects of *Stachys subnuda* extracts on MCF-7 breast cancer cells. Among the tested extracts, SSH exhibited the most potent cytotoxic activity, with the lowest IC_{50} value, suggesting its potential as an anticancer agent. Further mechanistic analyses revealed that SSH induces apoptosis via mitochondrial membrane depolarization, accompanied by an increased Bax/Bcl-2 ratio and caspase-7 activation. These findings indicate that SSH triggers mitochondrial-mediated apoptosis, a crucial pathway in cancer cell death.

Given the promising *in vitro* results, further studies are needed to identify the bioactive compounds responsible for the observed effects and to evaluate their efficacy in *in vivo* models. Additionally, the safety, pharmacokinetic properties, selectivity index, and time-dependent (48-72h) efficacy of SSH should be investigated to determine its therapeutic potential. Overall, our findings highlight *Stachys subnuda* as a valuable source for natural product-based anticancer research and encourage further exploration of its medicinal applications.

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Design of the study: PMT, AŞ
Acquisition of data for the study: İA, SKS, GAT
Analysis of data for the study: PMT, SKS, OO, İA
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REFERENCES

[1] Kamal N, Ilowefah MA, Hilles AR, Anua NA, Awin T, Alshwyeh HA, Aldosary SK, Jambocus NGS, Alosaimi AA, Rahman A, Mahmood S, Mediani A. Genesis and mechanism of some cancer types and an overview on the role of diet and nutrition in cancer prevention. Molecules. 2022;27(6):1794. https://doi.org/10.3390/molecules27061794

[2] Siegel RL, Kratzer TB, Giaquinto AN, Sung H, Jemal A. Cancer statistics, 2025. CA Cancer J Clin. 2025;75(1):10-45. https://doi.org/10.3322/caac.21871

[3] Lukasiewicz S, Czeczelewski M, Forma A, Baj J, Sitarz R, Stanislawek A. Breast cancer-epidemiology, risk factors, classification, prognostic markers, and current treatment strategies-an updated review. Cancers (Basel). 2021;13(17):4287. https://doi.org/10.3390/cancers13174287

[4] Henry NL, Ganz PA. Unintended negative consequences of managing chemotherapy toxicity. J Natl Cancer Inst. 2022;114(12):1572-4. https://doi.org/10.1093/jnci/djac174

[5] Alkhaifi M, Zhang E, Peera M, Jerzak K, Czarnota G, Eisen A, Roberts A, Carmona-Gonzalez CA, Pezo R, Gandhi S. Risk factors for treatment toxicity and high side effect burden among breast cancer survivors: a retrospective chart review. Cancers. 2025;17(2):328. https://doi.org/10.3390/cancers17020328

[6] Sitarek P, Merecz-Sadowska A, Sliwinski T, Zajdel R, Kowalczyk T. An in vitro evaluation of the molecular mechanisms of action of medical plants from the lamiaceae family as effective sources of active compounds against human cancer cell lines. Cancers (Basel). 2020;12(10):2957. https://doi.org/10.3390/cancers12102957

[7] Cox-Georgian D, Ramadoss N, Dona C, Basu C. Therapeutic and medicinal uses of terpenes. Medicinal Plants. 2019: 333-359.

[8] Pashova S, Karcheva-Bahchevanska D, Ivanov K, Ivanova S. Genus stachys-phytochemistry, traditional medicinal uses, and future perspectives. Molecules. 2024;29(22):5345. https://doi.org/10.3390/molecules29225345

[9] Babacan EY. Essential oil components of endemic stachys tundjeliensis kit tan & sorger (lamiaceae) and a chemotaxonomic approach. Journal of the Institute of Science and Technology. 2022;11(2):563-570. https://doi.org/10.21597/jist.1039366

[10] Ferrer-Gallego PP, Güner Ö. Lectotypification of stachys subnuda (lamiaceae). Phytotaxa. 2022;552(1):122-4. https://doi.org/10.11646/phytotaxa.552.1.12

[11] Satıl F, Açar M. Ethnobotanical use of Stachys L. (Lamiaceae) taxa in Turkey. International Journal of Nature and Life Sciences. 2020;4(2):66-86. https://doi.org/10.47947/ijnls.736452

[12] Sen A, Kurkcuoglu M, Bitis L, Dogan A, Baser KHC. Chemical composition of endemic Stachys subnuda Montbret & Aucher ex Benth. essential oil and its anti-inflammatory and antioxidant activities. Journal of Essential Oil Research. 2019;31(4):326-334. https://doi.org/10.1080/10412.905.2019.1567399

[13] Pejin B, Kojic V, Bogdanovic G. An insight into the cytotoxic activity of phytol at in vitro conditions. Nat Prod Res. 2014;28(22):2053-2056. https://doi.org/10.1080/14786.419.2014.921686

[14] de Alencar M, Islam MT, de Lima RMT, Paz M, Dos Reis AC, da Mata A, Filho J, Cerqueira GS, Ferreira PMP, JMC ES, Mubarak MS, Melo-Cavalcante AAC. Phytol as an anticarcinogenic and antitumoral agent: An in vivo study in swiss mice with DMBA-Induced breast cancer. IUBMB Life. 2019;71(2):200-212. https://doi.org/10.1002/iub.1952

[15] Di Sotto A, Romaniello D, Freddoni G, Abete L, Cocchiola R, Di Giacomo S, Altieri F, Mazzanti G, Eufemi M. New insights in the antitumor effects of β-caryophyllene in breast cancer cells: The role of cannabinoid and adrenergic systems. Annals of Oncology. 2018;29. https://doi.org/10.1093/annonc/mdy047.042

[16] Şen A, Göğer F, Dogan A, Bitis L. Two acylated isoscutellarein glucosides with anti-inflammatory and antioxidant activities isolated from endemic stachys subnuda montbret & aucher ex benth. Acta Chimica Slovenica. 2019:831-838. https://doi.org/10.17344/acsi.2018.4921

[17] İşcan G, Köse YB, Demirci B. Stachys Rupestris (Lamiaceae)'in uçucu yağ bileşimi ve antimikrobiyal etkileri. Anadolu University Journal of Science and Technology C – Life Science and Biotechnology. 2015;4(1):41-47. https://doi.org/10.18036/btdc.62075

[18] Kim CW, Choi KC. Potential roles of iridoid glycosides and their underlying mechanisms against diverse cancer growth and metastasis: Do they have an inhibitory effect on cancer progression? Nutrients. 2021;13(9):2974. https://doi.org/10.3390/nu13092974

[19] Senkardes S, Atlihan I, Cayir E, Mega Tiber P, Orun O, Nigiz S, Ozkul C, Gunduz MG, Kucukguzel SG. Synthesis and evaluation of novel metacetamol derivatives with hydrazone moiety as anticancer and antimicrobial agents. Chem Biodivers. 2023;20(8):e202300766. https://doi.org/10.1002/cbdv.202300766

[20] Atlıhan İ, Koçyiğit Sevinç S, Orun O, Yılmaz Ö, Güniz Küçükgüzel Ş, Mega Tiber P. Effect of flurbiprofen derivative (SGK597) on cell proliferation and apoptosis of breast cancer cell lines. İstanbul Journal of Pharmacy. 2022;52(3):265-270. https://doi.org/10.26650/IstanbulJPharm.2022.109.4112

[21] Wang C, Youle RJ. The role of mitochondria in apoptosis. Annu Rev Genet. 2009;43:95-118. https://doi.org/10.1146/annurev-genet-102.108.134850

[22] Gibellini L, Bianchini E, De Biasi S, Nasi M, Cossarizza A, Pinti M. Natural compounds modulating mitochondrial functions. Evid Based Complement Alternat Med. 2015;527209:1-13. https://doi.org/10.1155/2015/527209

[23] Arokia Femina T, Barghavi V, Archana K, Swethaa NG, Maddaly R. Non-uniformity in in vitro drug-induced cytotoxicity as evidenced by differences in IC(50) values – implications and way forward. J Pharmacol Toxicol Methods. 2023;119:107238. https://doi.org/10.1016/j.vascn.2022.107238

[24] Berrouet C, Dorilas N, Rejniak KA, Tuncer N. Comparison of drug inhibitory effects (IC_{50}) in monolayer and spheroid cultures. Bull Math Biol. 2020;82(6):68. https://doi.org/10.1007/s11538.020.00746-7

[25] Kongul Safak E, Seker Karatoprak G, Dirmenci T, Duman H, Kucukboyaci N. Cytotoxic effects of some Nepeta species against breast cancer cell lines and their associated phytochemical properties. Plants (Basel). 2022;11(11):1427. https://doi.org/10.3390/plants11111427

[26] Panahi Kokhdan E, Sadeghi H, Danaei N, Sadeghi H. Cytotoxic effect of hydroalcoholic extract of Stachys setifera on MCF-7 human breast cancer cell line. Armaghane Danesh. 2021;26(1):33-44. https://doi.org/10.52547/armaghanj.26.1.33

[27] Slimani W, Maioli M, Cruciani S, Zerizer S, Santaniello S, Kabouche Z, Coradduzza D, Chessa M, Fancello S, Migheli R, Serra PA, D'Hallewin G. Antioxidant, anti-inflammatory and anti-proliferative properties of Stachys circinata on HepG2 and MCF7 cells. Plants (Basel). 2023;12(12):2272. https://doi.org/10.3390/plants12122272

[28] Vali A, Soleimani Badie M, Dastan D, Moradkhani S. Cytotoxicity of the extracts of Lamiaceae plant species against a breast cancer cell line. Iranian Journal of Toxicology. 2023;17(4):35-41. https://doi.org/10.61186/ijt.17.4.35

[29] Bonta RK. Dietary phenolic acids and flavonoids as potential anti-cancer agents: current state of the art and future perspectives. Anticancer Agents Med Chem. 2020;20(1):29-48. https://doi.org/10.2174/187.152.0619666.191.019112712

[30] Rabi T, Bishayee A. Terpenoids and breast cancer chemoprevention. Breast Cancer Res Treat. 2009;115(2):223-39. https://doi.org/10.1007/s10549.008.0118-y

[31] Eruygur N, Ataş M, Çevir Ö, Tekin M. Investigating of phytochemicals, antioxidant, antimicrobial and proliferative properties of different extracts of Thymus spathulifolius Hausskn. and Velen. endemic medicinal plant from Sivas, Turkey. International Journal of Secondary Metabolite. 2017:155-166. https://doi.org/10.21448/ijsm.369775

[32] Abubakar AR, Haque M. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. J Pharm Bioallied Sci. 2020;12(1):1-10. https://doi.org/10.4103/jpbs.JPBS_175_19

[33] Cravotto C, Fabiano-Tixier AS, Claux O, Abert-Vian M, Tabasso S, Cravotto G, Chemat F. Towards substitution of hexane as extraction solvent of food products and ingredients with no regrets. Foods. 2022;11(21):3412. https://doi.org/10.3390/foods11213412

[34] Carré P, Berthold S, Piofczyk T, Bothe S, Hadjiali S. Solvent solutions: comparing extraction methods for edible oils and proteins in a changing regulatory landscape. General introduction. Ocl. 2025;32:3. https://doi.org/10.1051/ocl/2024034

[35] Zapor L, Skowron J, Golofit-Szymczak M. The cytotoxicity of some organic solvents on isolated hepatocytes in monolayer culture. Int J Occup Saf Ergon. 2002;8(1):121-129. https://doi.org/10.1080/10803.548.2002.11076520

[36] Naseri MH, Mahdavi M, Davoodi J, Tackallou SH, Goudarzvand M, Neishabouri SH. Up regulation of Bax and down regulation of Bcl2 during 3-NC mediated apoptosis in human cancer cells. Cancer Cell Int. 2015;15:55. https://doi.org/10.1186/s12935.015.0204-2

[37] Jelinek M, Balusikova K, Schmiedlova M, Nemcova-Furstova V, Sramek J, Stancikova J, Zanardi I, Ojima I, Kovar J. The role of individual caspases in cell death induction by taxanes in breast cancer cells. Cancer Cell Int. 2015;15(1):8. https://doi.org/10.1186/s12935.015.0155-7

[38] Twiddy D, Cohen GM, Macfarlane M, Cain K. Caspase-7 is directly activated by the approximately 700-kDa apoptosome complex and is released as a stable XIAP-caspase-7 approximately 200-kDa complex. J Biol Chem. 2006;281(7):3876-3888. https://doi.org/10.1074/jbc.M507393200

[39] Janicke RU. MCF-7 breast carcinoma cells do not express caspase-3. Breast Cancer Res Treat. 2009;117(1):219-21. https://doi.org/10.1007/s10549.008.0217-9

[40] Bouchouka RL, Kabouche Z, Defondaumiere M, Debiossat M, Ghezzi C, Riou L, Taha TH, Boufahja F, Bendif H, Garzoli S. LC-HRMS/MS-guided profiling and biological evaluation of Stachys duriaei extracts: Anticancer and vasorelaxant mechanisms via apoptosis and endothelium-dependent pathways. Molecules. 2025;30(17):3570. https://doi.org/10.3390/molecules30173570

[41] Hosseinzadeh L, Hajmomeni P, Salehi M, Modarresi M, Jalilian F. Anti-proliferative activity and apoptosis induction of extracts and fractions of Stachys lavandulifolia on lung (H1299), ovarian (A2780) and breast (MCF-7) human cancer

cell lines. Iran J Pharm Res. 2025;24(1):e152370. https://doi.org/10.5812/ijpr-152370

[42] Shakeri A, Hafezian T, Kusz N, Hohmann J, Boozari M, Mottaghipisheh J, Emami SA, Tayarani-Najaran Z, Asili J. Cytotoxicity, apoptosis inducing activity and Western blot analysis of tanshinone derivatives from Stachys parviflora on prostate and breast cancer cells. Mol Biol Rep. 2022;49(9):8251-8258. https://doi.org/10.1007/s11033.022.07541-8