Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2024;49(1):31-39
DOI: 10.17826/cumj.13860120

RESEARCH

Saponin rich *Gypsophila eriocalyx* Boiss methanolic extract (GEME) exhibit anticancerogenic effect against neuroblastoma cancer cell line (SH-SY5Y)

Saponinden zengin *Gypsophila eriocalyx* Boiss metanolik ekstraktı (GEME) nöroblastom kanser hücre hattı (SH-SY5Y) üzerinde antikanserojenik etki göstermektedir

Serap Niğdelioğlu Dolanbay[1]

[1]Gazi University, Ankara, Turkey

Abstract

Purpose: Herein, we sought to determine whether of saponin rich *Gypsophila eriocalyx* methanol extract (GEME) would exhibit any anti-carcinogenic activity on neuroblastoma cancer cell line (SH-SY5Y).

Materials and Methods: We therefore determined GEME's saponin composition using LC-MS analysis, its impact on cell viability using MTT analysis, flow cytometry, and to find out its impact on apoptosis using qRT-PCR analysis.

Results: In the LC-MS analysis we determined that GEME contained high amounts of saponin (0.05-0.48 µg/g). We determined that GEME had an IC_{50} dose of 100 µg/mL at 48 hours. GEME had the effect of substantially increasing the percentage of apoptotic cells (5.19% and 65.21%) and disruption of mitochondria (46.18%). We also demonstrated that *BCL2* gene expression (2.76 fold) was significantly reduced than that of the control while *BAX* (2.21 fold), *CASP3* (2.43 fold), *CASP7* (2.98 fold), *CASP8* (2.23 fold), *CASP9* (2.78 fold), and *CYCS* (2.12 fold) genes were expressed significantly higher than those of the control.

Conclusion: Considering the findings, it becomes clear that saponin-rich GEME stands out as a significant anticarcinogenic agent. Its remarkable efficacy is demonstrated through its capabilities to notably reduce cell viability, effectively trigger apoptosis, and significantly increase the rate of mitochondrial disruption in cancer cells.

Keywords: Anticancerogenic effect; *Gypsophila eriocalyx* Boiss; methanolic extract; neuroblastoma cancer cell line (SH-SY5Y)

Öz

Amaç: Bu çalışmada saponinden zengini *Gypsophila eriocalyx* metanol ekstraktı (GEME)'nın nöroblastom kanserr hücre hattı (SH-SY5Y) üzerinde herhangi bir anti-karsinojenik aktivite gösterip göstermediğini belirlemeyi amaçladık.

Gereç ve Yöntem: Bu nedenle GEME'nin saponin kompozisyonunu LC-MS analizi kullanarak, hücre canlılığı üzerindeki etkisini MTT analizi, apoptozis üzerindeki etkisini akış sitometrisi ve qRT-PCR analizi ile belirledik.

Bulgular: LC-MS analizinde, GEME'nin yüksek miktarda saponin (0,05-0,48 µg/g) içerdiğini belirledik. GEME'nin 48 saatte 100 µg/mL IC_{50} dozuna sahip olduğunu belirledik. GEME'nin apoptotik hücrelerin yüzdesini (%5,19 ve %65,21) önemli ölçüde arttırma ve mitokondrilerin bozulmasına (%46,18) etkisi oldu. Ayrıca, *BCL2* (2,76 kat) gen ekspresyonunun kontrol grubundan önemli ölçüde düşük olduğunu; *BAX* (2,21 kat), *CASP3* (2,43 kat), *CASP7* (2,98 kat), *CASP8* (2,23 kat), *CASP9* (2,78 kat) ve *CYCS* (2,12 kat) genlerinin ise kontrol grubundan anlamlı derecede yüksek olduğunu gösterdik.

Sonuç: Bulgulara bakıldığında saponin açısından zengin GEME'nin önemli bir antikarsinojenik ajan olarak öne çıktığı ortaya çıkmaktadır. Dikkate değer etkinliği, hücre canlılığını önemli ölçüde azaltma, apoptozu etkili bir şekilde tetikleme ve kanser hücrelerinde mitokondriyal bozulma oranını önemli ölçüde artırma yetenekleriyle kanıtlanmıştır.

Anahtar kelimeler: Antikarsinojenik etki, *Gypsophila eriocalyx* Boiss, metanol ekstraktı, nöroblastom kanser hücre hattı (SH-SY5Y)

Address for Correspondence: Serap Niğdelioğlu Dolanbay, Gazi University, Faculty of Science, Department of Biology, Ankara, Turkey E-mail: serapdolanbay@gazi.edu.tr
Received: 04.11.2023 Accepted: 31.12.2023

INTRODUCTION

Neuroblastoma (NB) is among the pediatric extracranial solid tumors with the highest incidence, which originates from neural crest progenitor cells residing in the sympathetic nervous system. It manifests with mass lesions involving the adrenal glands and/or sympathetic ganglia[1]. NB is responsible for a tenth of all cancer cases diagnosed in the childhood, mainly occurring in children younger than 5 years of age. Its has a huge impact on cancer mortality in childhood, especially in children with high-risk relapses resistant to chemotherapy, which lower their survival rate to well lower than 40%[2]. Although much effort has been made to reduce toxicity of NB treatments and rendering them curative, they remain considerably toxic and far from being curative[3]. Thus, there is an increased interest in discovering natural herbal products to prevent and treat NB[4].

Gypsophila L. species belong to the Caryophyllaceae family and found chiefly in Mediterranean and Iran-Turan areas. *Gypsophila* has 56 species in 10 sections in Turkey, where 33 species are endemic. It has a great importance for the biodiversity of Turkey[5]. *Gypsophila* taxa possesses a variety of biological properties which have been exploited in folk remedies and in food manufacturing[6]. In a study by Altay et al.[6], in its extracts were found to contain abondant saponins, which are known to exert potent antioxidant and antiproliferative effects on various cancer cell lines (HepG2, HT-29, and MCF-7). Furthermore, the extracts of these taxa were found to have significant antidiabetic and anticholinergics effects. Zhang et al.[7] demonstrated a high saponin content in *G. oldhamiana* root extract (TGOE) using LC-electrospray ionization (ESI)-M^{Sn}. Various anti-proliferative effects and apoptotic activities of TGOE on SMMC-7721 and L02 cell lines were also evaluated. Nevertheless, it has been not studied whether *Gypsophila eriocalyx* species endemic to Turkey would exert any anti-cancerogenic effect on the SH-SY5Y cell line.

Herein, we attempted to delineate the saponin content of *Gypsophila eriocalyx* methanolic extraxt (GEME) as well as to evaluate its activities suppressing cell viability and promoting apoptosis. In this work, GEME's saponin content, cell viability-suppressing properties, and impacts on apoptosis-related gene expression levels are all assessed for the first time.

MATERIALS AND METHODS

This research was conducted at Gazi University, one of Turkey's leading educational and research institutions. The university's state-of-the-art laboratories and extensive research facilities enable comprehensive studies across various scientific disciplines. Gazi University maintains high standards in rigorous experimental protocols, meticulous record management, and ensuring data integrity, thereby ensuring the reliability of produced files and data through these practices. I am Serap Niğdelioğlu Dolanbay, leading this study. My academic career is enriched with advanced research, and I have extensive experience in areas like cancer research and pharmacology. In this study, I've applied my expertise to make significant contributions. As a doctor at Gazi University, I've played a crucial role in the success of this research.

Plant material

The natural habitat of *Gypsophila eriocalyx* Boiss is located in Beypazarı, Ankara, Turkey, where it was collected in its blooming phase in August 2011. The species confirmation was conducted by Prof. Dr. Zeki AYTAÇ, a specialist in plant taxonomy from the Biology Department at Gazi University in Ankara, Turkey. For future reference and study, a representative sample has been conserved at the Gazi University Herbarium in Ankara, Turkey, with the voucher identification number ZA-10435-41.

Preparation of the methanolic extract

Soxhlet apparatus with methanol (HPLC grade) was used to extract dried and powdered plant material at a weight of 30 g in a reaction at 60 °C for 4 hours. Then, a rotary evaporator (Heidolph) was used to filter and concentrate the *Gypsophila eriocalyx* methanolic extract (GEME) under vacuum at 80 °C; then the extract was left to dry and stored at 4 °C to be used within 1 week at maximum.

Determination of saponin composition

Agilent 6460 Triple Quadrupole System (ESI + Agilent Jet Stream) combined with Agilent 1200 Series HPLC was used for the analysis. Data regarding the amount of saponins in the GEME was

determined with their respective concentration vs. peak area calibration curves.

Determination of cell viability

The SH-SY5Y human neuroblastoma cell line (ATCC CRL-2266) was procured from the Sap Institute in Ankara, Turkey. Cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM) enriched with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, and 1% L-glutamine. Culturing was carried out at a temperature of 37°C within a humidified atmosphere containing 5% CO_2 and 95% air. The growth medium was refreshed every two to three days until the cells neared full confluency. GEME's effect on cell viability in cancer cells was assessed on SH-SY5Y. SH-SY5Y cells were plated in 96-well plates at a density of 10^4 cells per well and incubated at 37 °C with 5% carbon dioxide (CO_2). Following the completion of incubation for 24, 48, and 72 hours, GEME (0, 10, 25, 50, 100, 250, 500, and 1000 µg/mL) was used to treat the cells. After GEME was removed from the wells, PBS was used to irrigate the cells, and the wells were supplied with fresh medium. MTT assay was then used to find cell viability. Every microwell was provided with a total of 20 µL of 0.5% MTT in phosphate buffered saline (PBS). The cells were incubated for 4 hours; afterwards, the medium was discarded and formazan crystals were dissolved by adding 200 µL dimethyl sulfoxide (DMSO). An ELISA microplate reader (BioTek) at 570 nm measured the absorbance level to calculate viability as the ratio (shown as a percentage) of absorbance level of treated cells to untreated cells serving as control.

Flow cytometric detection of apoptotic cells

Following the completion of GEME treatment, the cells were collected and irrigated with PBS. They were then subjected to resuspension in 1x annexin-binding buffer (Abcam) at a concentration 1×10^6 cells/mL. Each 100 µL of the cell suspension (up 1×10^6 cells) was added 5 µL of the annexin V-fluorescein isothiocyanate (FITC) (Abcam) and 5 µL of propidium iodide (PI) (Abcam). Cell incubation was then performed in the dark at room temperature for a duration of 15 minutes, which was followed by the addition of 400 µL of 1x annexin-binding buffer. Afterwards, analysis of the cells was carried out with the help of ACEA NovoCyte flow cytometry. Ten thousand cells per sample were analyzed, followed by the determination of the percentages of live, apoptotic, and necrotic cells by the ACEA NovoExpress software.

Flow cytometric detection of mitochondrial membrane potential (Δψm)

After GEME treatment was carried out as mentioned above, the cells were supplemented with tetraethylbenzimidazolylcarbo-cyanine iodide (JC-1) (100 µL) (Cayman Chemical) (up 1×10^6 cells). When the culture at 37 °C was completed after 30 minutes, the cells were caught and removed by pipetting, irrigated with PBS for two times, and analyzed with the ACEA NovoCyte flow cytometer. Ten thousand cells per sample were analyzed, followed by the determination of the percentages of intact and distrupted mitochondria by the ACEA NovoExpress software.

qRT-PCR

Total RNA was isolated from SH-SY5Y cells using the RNeasy Mini Kit (Qiagen), and cDNA was synthesized using the QuantiTect Reverse Transcription Kit (Qiagen), following the protocols provided by the manufacturer. To assess the impact of a 48-hour GEME treatment on gene expression, qRT-PCR was employed, measuring the levels of *BAX*, *BCL2*, *CASP3*, *CASP7*, *CASP8*, *CASP9*, and *CYCS* using specific primers for each gene. β-actin served as the normalization control. The PCR conditions were as follows: an initial denaturation at 95°C for 5 minutes, followed by 40 cycles of 10 seconds at 95°C for denaturation, 30 seconds at 60°C for annealing, and 30 seconds at 72°C for extension. Relative gene expression changes were calculated using the $2^{-\Delta\Delta CT}$ method, and the results were expressed in terms of fold change.

Statistical analysis

In my study, all experimental procedures were meticulously conducted in triplicates to ensure reliability and reproducibility of the data. The outcomes of these experiments were then presented as the mean value derived from these three independent replicates. To express the variability and dispersion within the data, we reported the results as mean ± standard deviation (SD), providing a clear indication of the spread of the data around the mean. For the purpose of statistical analysis, I utilized the SPSS software, version 11.0 (SPSS Inc.), known for its robust statistical tools and user-friendly interface.

This software facilitated the application of the student's t-test, a widely recognized statistical method, in my study. The student's t-test was specifically employed to compare the means between two distinct groups in my experiments. This comparison was crucial for evaluating the significance of the differences observed in the experimental data, thereby contributing to the validity and strength of my overall findings.

RESULTS

Saponin composition of GEME

GEME's saponin composition was determined using LC-MS/MS analysis. We found that gypsogenin and quillaic acid had the highest and lowest quantities, respectively in the saponin composition of GEME (Table 1).

Table 1. Saponin composition and quantity of GEME.

Saponin composition	Control	Quantity (µg/g)
Gypsogenin	0.00 ± 0.00	0.48 ± 0.03*
Gypsogenic acid	0.00 ± 0.00	0.08 ± 0.02*
Hederagenin	0.00 ± 0.00	0.19 ± 0.07*
Nebulosides	0.00 ± 0.00	0.27 ± 0.01*
Quillaic acid	0.00 ± 0.00	0.05 ± 0.03*

Values represent averages ± SD for triplicate experiments. *Student's t-test, $p<0.05$, compared with the control group.

Effect of GEME on Cell Viability

The MTT assay was utilized to assess the impact of GEME on the viability of SH-SY5Y cells. GEME was administered to the SH-SY5Y cell line at eight different dosages (0, 10, 25, 50, 100, 250, 500, and 1000 µg/mL) over three time intervals (24, 48, and 72 hours). Viability outcomes were referenced against that of the control cells. There was a notable enhancement in the influence of GEME on SH-SY5Y cell viability correlating with increased concentration and longer exposure duration ($p<0.05$). The IC_{50} value of GEME was determined to be 100 µg/mL at the 48-hour time point. Subsequent experiments were conducted with this IC_{50} value (refer to Fig 1).

Effect of GEME on apoptotic cells using flow cytometry

In the flow cytometric analyses of SH-SY5Y cells, which were stained with annexin V and propidium iodide, it was revealed that the early apoptotic cells, late apoptotic cells, and necrotic cells in SH-SY5Y cell line were significantly increased in percentage by GEME, as compared with the control cells ($p<0.05$) (Fig 2 and Table 2).

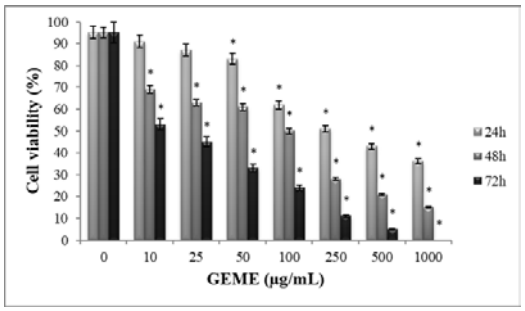

Figure 1. Cell viability of various concentrations (0, 10, 25, 50, 100, 250, 500, and 1000 µg/mL) of GEME on SH-SY5Y cells for 24, 48 and 72 h.

Values represent averages ± SD for triplicate experiments. *p < 0.05 Student's t-test, compared with the control group.

Table 2. Effect of GEME on live, early and late apoptotic, and necrotic cells (%) in SH-SY5Y cells using flow cytometry.

Type of Cells	Control (%)	GEME (%)
Live	85.59	12.19*
Early apoptotic	3.49	5.19*
Late apoptotic	3.32	65.21*
Necrotic	7.59	16.41*

*Student's t-test, $p<0.05$, compared with the control group.

Effect of GEME on Δψm

In the flow cytometric analyses of SH-SY5Y cells, which were stained with JC-1, it was revealed that the distrupted mitochondria in SH-SY5Y cell line were significantly increased in percentage by GEME, as compared with the control cells ($p<0.05$) (Fig 3 and Table 3).

Table 3. Effect of GEME intant and distrupted mitochondria (%) in SH-SY5Y cells using flow cytometry.

Types of mitochondria	Control (%)	GEME (%)
Intact	91.51	53.70*
Distrupted	8.46	46.18*

*Student's t-test, $p<0.05$, compared with the control group.

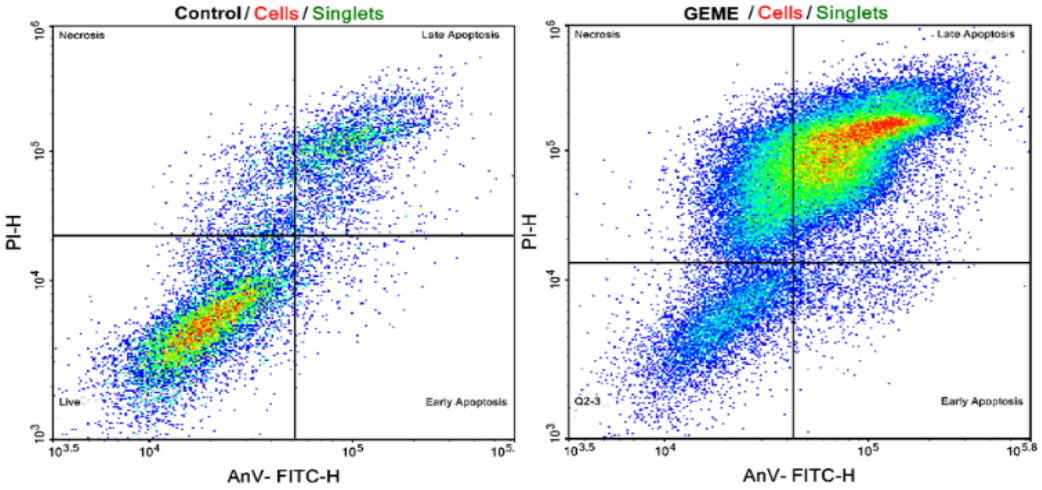

Figure 2. Effect of GEME on apoptosis in SH-SY5Y cells using flow cytometry.

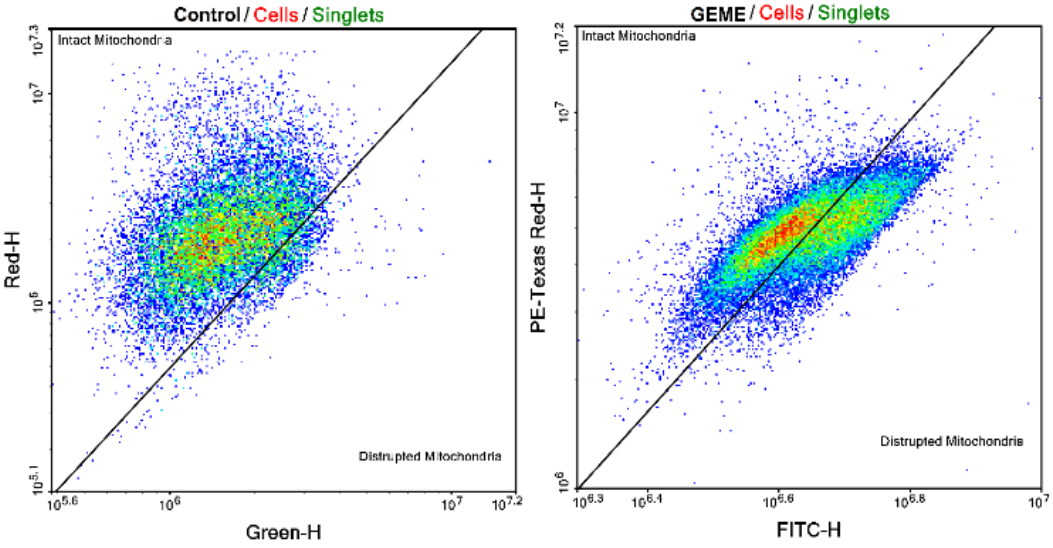

Figure 3. Effect of GEME on the loss of Δψm and the release of mitochondrial cytochrome *c* into the cytosol in SH-SY5Y cells using flow cytometry.

Effect of GEME on Gene Expression Levels of Related to Apoptosis

Using qRT-PCR, changes in the expression levels of genes involved in apoptosis were measured in the SH-SY5Y cell line after a 48-hour period, which included *BAX*, *BCL2*, *CASP3*, *CASP7*, *CASP8*, *CASP9*, and *CYCS*. A substantial decrease in *BCL2* gene expression was observed when compared to the control ($p<0.05$). In contrast, expressions of the *BAX*, *CASP3*, *CASP7*, *CASP8*, *CASP9*, and *CYCS* genes significantly increased relative to the control ($p<0.05$) (see Fig 4).

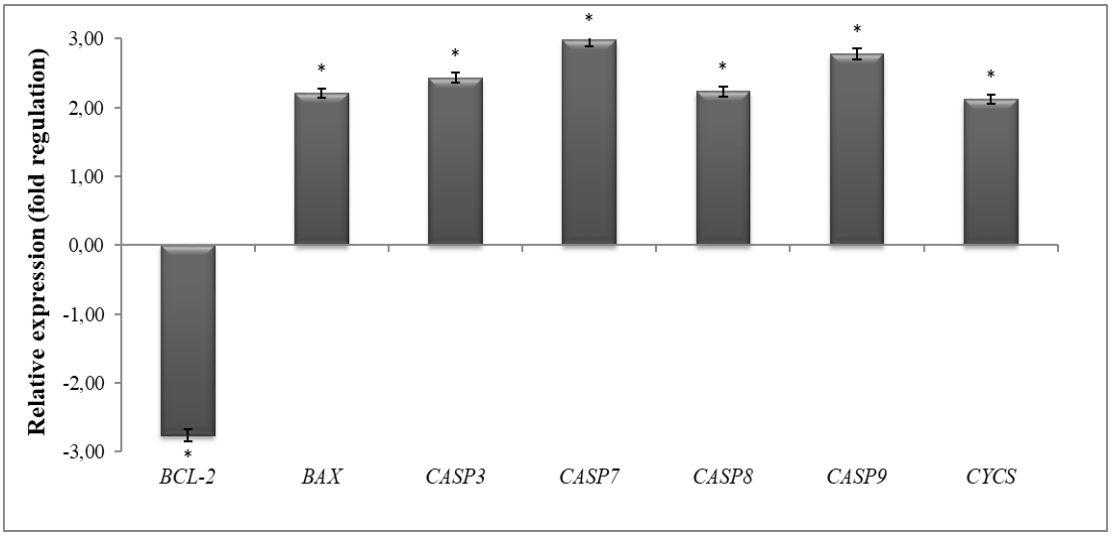

Figure 4. Effect of GEME (100 µg/mL) on relative expression levels of genes (*BCL-2*, *BAX*, *CASP3*, *CASP7*, *CASP8*, *CASP9*, and *CYCS*) role in apoptosis on SH-SY5Y cells for 48 h. Values represent averages ± SD for triplicate experiments. *Student's t-test, $p<0.05$, compared with the control group.

DISCUSSION

The study I conducted delves into the rich and complex world of saponins within the *Gypsophila* genus, with a specific focus on the *Gypsophila eriocalyx* methanol extract (GEME). Saponins, a diverse family of natural compounds widely distributed in various plants, are particularly abundant in the *Gypsophila* species, especially in their underground parts. This abundance makes these species a valuable subject for phytochemical and pharmacological research[8]. In my research, I specifically identified and quantified the saponin composition of GEME using LC-MS/MS analysis, a highly precise and accurate method for chemical analysis. This analysis revealed gypsogenin as the predominant saponin structure in GEME, along with significant amounts of hederagenin, gypsogenic acid, quillaic acid, and nebulosides. The revelation that 15 to 20 percent of the *Gypsophila* taxa content consists of saponin glycosides underscores the richness of these compounds in the genus. Furthermore, the presence of gypsogenine, a pentacyclic compound, along with a variety of sugars including galactose, xylose, arabinose, fructose, and rhamnose, points to a complex phytochemical profile that could have significant biological implications[9]. The findings from my study are consistent with existing literature, including the research by Yücekutlu and Bildacı on *G. simonii*[10]. This species, an endemic plant, was studied for its unripe saponins which were extracted from its roots and analyzed for their chemical and physical properties. The alignment of our study with these findings further validates the widespread presence of saponins in the *Gypsophila* genus and particularly in the *G. eriocalyx* species. This agreement with the literature not only reaffirms the saponin content in *G. eriocalyx* but also enhances our understanding of the chemical diversity within the *Gypsophila* genus. The comprehensive analysis of GEME's saponin composition contributes to the broader field of natural product research and opens up potential avenues for exploring these compounds in various applications, including their pharmacological and therapeutic uses. The detailed characterization of these saponins paves the way for future studies to investigate their biological activities and potential benefits in medicine, particularly in the development of new treatments and therapies.

The significance of natural compounds, particularly saponin-rich herbal extracts, in the development of anticancer drugs cannot be overstated. The inherent properties of these compounds in combating cancer have been substantiated through various studies[11]. The study by Chen et al.[12] highlights the anticarcinogenic properties of diosgenin, a type of saponin. Their findings that diosgenin not only

inhibits tumor proliferation but also promotes apoptosis in tumor cells is a critical contribution to understanding the therapeutic potential of saponins. The ability to induce programmed cell death in cancer cells, while limiting their growth, is a cornerstone in effective cancer treatment strategies. Furthermore, their research extends to understanding the impact of diosgenin on the migratory and invasive capabilities of the human prostate cancer PC-3 cell line, which is crucial for understanding how these compounds might inhibit metastasis, a key factor in cancer progression. Building on this foundation, the exploration of GEME, which is rich in saponins, in relation to its effects on the SH-SY5Y cell line, opens up new avenues in cancer research. This specific cell line, derived from a neuroblastoma, is an excellent model for studying neuronal cell death and neurotoxicity, making it relevant for investigating the neuroprotective or neurotoxic properties of compounds. The potential anticancer effects of GEME on this cell line could provide insights not only into its efficacy in inhibiting tumor growth and inducing apoptosis but also in understanding its broader impact on neural cells. This approach of studying GEME's effects on the SH-SY5Y cell line could lead to a better understanding of the mechanisms through which saponins exert their anticarcinogenic properties. Additionally, it could also contribute to the development of more targeted and effective anticancer therapies, especially for cancers that affect the nervous system or exhibit neurotoxic side effects. In summary, the exploration of GEME's antitumor potential represents a significant step forward in the realm of natural compound-based cancer therapeutics. It not only reinforces the importance of saponins as valuable components in anticancer drug development but also underscores the need for continued research in this area to fully harness their therapeutic potential.

The study on the effects of saponin-rich GEME on cell viability, particularly focusing on the SH-SY5Y cell line, is an important contribution to the field of cancer research. The use of the MTT assay in this study is a key methodological highlight, as it provides a reliable and quantitative assessment of cell viability and proliferation. The determination of the IC_{50} value, which stands for the concentration of the substance required to inhibit cell viability by 50%, is a crucial aspect of this research. For the SH-SY5Y cell line, the IC_{50} dose of GEME was found to be 100 µg/mL at 48 hours. This finding is significant as it provides a quantitative measure of the extract's efficacy and potency in inhibiting the growth of cancer cells. Comparatively, the study by Altay et al.[6] on the antiproliferative properties of both methanol and water extracts of *G. eriocalyx* in different cell lines (HepG2 (IC_{50}: 269 µg/mL, 856 µg/mL, 48 h), HT29 (IC_{50}: 170 µg/mL, 644 µg/mL, 48 h), and MCF-7 (IC_{50}: 381 µg/mL, 814 µg/mL, 48 h) reveals varying degrees of effectiveness, with methanol extract demonstrating a greater effect than the water extract. This variation in IC_{50} values across different cell lines underscores the specificity of GEME's action and its varying degrees of effectiveness depending on the cellular context. Additionally, the study by Lin et al.[13] on isoorientin, a compound obtained from *G. elegans*, further expands the understanding of the cytotoxic effects of *Gypsophila*-derived compounds. The reported increase in LDH levels, a marker of cell damage and cytotoxicity, along with varying IC_{50} values over different incubation periods, provides insights into the time-dependent efficacy of these compounds. Moreover, the findings by Rad et al.[14] regarding the chloroform fractions of *G. bicolor* and *G. ruscifolia* highlight the cytotoxicity of these extracts on MCF-7 cells, with a significant IC_{50} value of less than 100 µg/mL. However, the absence of cytotoxic effects on A549 or AGO-1522 cells at the tested concentrations indicates a selective toxicity profile, which is crucial for minimizing side effects in potential therapeutic applications. The alignment of these results with existing literature strengthens the credibility of the findings and suggests a promising avenue for further research. The use of advanced techniques like flow cytometry and qRT-PCR in future studies would enable a deeper understanding of the mechanisms through which saponin-rich GEME acts as an anticarcinogenic agent. Such comprehensive studies could unveil the molecular pathways involved, potentially leading to the development of targeted cancer therapies.

The research on the anticarcinogenic properties of saponin-rich GEME presents a compelling case for the potential use of natural compounds in cancer treatment. My study's findings, highlighting GEME's capacity to disrupt cell viability, enhance apoptosis, and increase the disruption of mitochondria, are significant contributions to this field. The use of flow cytometry and qRT-PCR in my study provides a robust and detailed understanding of these cellular processes at a molecular level. The triggering of the intrinsic apoptotic pathway by GEME, as evidenced by the increase in pro-apoptotic genes such as *BAX*, *CASP3*, *CASP7*, *CASP8*, *CASP9*, and *CYCS* and the

decrease in the anti-apoptotic gene *BCL2*, aligns well with the broader body of research on saponins. This mechanistic insight into how GEME induces apoptosis, particularly through mitochondrial mediation, is crucial for understanding its potential as a therapeutic agent. Furthermore, your reference to the study by Lin et al.[13] on *Gypsophila elegans* isolates (GEI) and its effect on HepG2 cells sheds light on the broader implications of saponin-rich compounds in cancer therapy. The regulation of cell cycle-related genes and the arrest of the cell cycle at the G1 phase by GEI highlight a critical mechanism by which these compounds can control tumor proliferation. The production of reactive oxygen species (ROS) and subsequent downregulation of matrix metalloproteinase (MMP) activity, as found in other studies, are important factors in the anticancer action of saponins. These processes, coupled with the release of cytochrome c and the increase in the proteolytic activities of CASP3 and CASP9, indicate a complex interaction of molecular events leading to cancer cell death. Moreover, the research by Tian et al.[15] on *Gypsophila oldhamiana* gypsogenin (GOG) demonstrates its effectiveness in reducing the growth and spread of Lewis lung cancer. This effect, attributed to the inhibition of tumor angiogenesis and the induction of apoptosis, further underscores the potential of *Gypsophila*-derived saponins in cancer control. In Liu et al.'s [16] study, the impact of GOG on the expression of VEGF and MMP-9 proteins, along with the upregulation of caspase-3 and Bax proteins, aligns with your findings on GEME.

The findings from my study about GEME, which is rich in saponins, are particularly noteworthy for their implications in cancer treatment. The extract's effectiveness as an anticarcinogenic agent is marked by its multi-faceted approach to combating cancer cells. First and foremost, GEME disrupts cell viability, indicating its potent cytotoxic effects on cancerous cells. This disruption of cell viability is a critical step in preventing the proliferation of cancer cells and is a desirable attribute in potential cancer therapies. Furthermore, GEME's ability to induce apoptosis, the programmed cell death vital for eliminating malignant cells, is particularly significant. The promotion of apoptosis is a key strategy in cancer treatment, as it helps in selectively targeting and eradicating cancer cells. The increased percentage of disrupted mitochondria observed in my study suggests that GEME causes mitochondrial dysfunction, an important trigger for the apoptotic process in cancer cells. This mitochondrial disruption is often a targeted approach in anticancer therapies, given its crucial role in cell survival and death. Perhaps most strikingly, my research advocates that GEME activates the mitochondria-mediated intrinsic apoptotic pathway. This pathway is one of the main mechanisms through which cells initiate apoptosis in response to internal stress signals, such as DNA damage or oxidative stress. The increase in pro-apoptotic genes like *BAX*, *CASP3*, *CASP7*, *CASP8*, *CASP9*, and *CYCS*, coupled with the decrease in the anti-apoptotic gene *BCL2*, is indicative of a shift towards cell death and away from survival. These molecular changes orchestrated by GEME highlight its potential mechanism of action in inducing apoptosis in cancer cells. In summary, the ability of GEME to act on multiple fronts – inhibiting cell viability, promoting apoptosis, causing mitochondrial disruption, and modulating key apoptotic genes – positions it as a promising candidate in the search for effective anticarcinogenic agents. These findings not only contribute to the growing body of research supporting the use of natural compounds in cancer therapy but also pave the way for further studies to explore and harness the full potential of GEME in oncological applications.

The present study provides valuable insights into the anticancerogenic potential of *Gypsophila eriocalyx* Boiss methanolic extract (GEME) against the SH-SY5Y neuroblastoma cell line. However, there are several limitations to be acknowledged. Firstly, the results are based solely on *in vitro* experiments with a specific cell line, which may not fully represent the complex interactions and environment within a living organism. This limits the generalizability of our findings to *in vivo* scenarios. Additionally, while GEME has shown promising results in these specific cellular models, its efficacy and safety in humans have not been evaluated. Future studies involving animal models and eventually clinical trials in humans are necessary to validate these findings. Lastly, the specific mechanisms by which GEME exerts its anticancerogenic effects have not been fully elucidated, and further molecular studies are needed to understand these pathways.

Author Contributions: Concept/Design : SND; Data acquisition: SND; Data analysis and interpretation: SND; Drafting manuscript: SND; Critical revision of manuscript: SND; Final approval and accountability: SND; Technical or material support: SND; Supervision: SND; Securing funding (if available): n/a.
Ethical Approval: None. This study, focused on investigating the effects of plant extract on cell line, does not require ethical approval as it is conducted entirely on in vitro cell cultures. Studies involving cell lines are exempt from ethical approval processes as they do not involve live human or animal subjects.
Peer-review: Externally peer-reviewed.

Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
Acknowledgement: I would like to thank Prof. Dr. Belma Aslım for providing *Gypsophila eriocalyx* Boiss methanolic extract.

REFERENCES

1. Wienke J, Dierselhuis MP, Tytgat GA, Künkele A, Nierkens S, Molenaar JJ. The immune landscape of neuroblastoma: Challenges and opportunities for novel therapeutic strategies in pediatric oncology. Eur J Cancer. 2021;144:123-50.
2. Ponzoni M, Bachetti T, Corrias MV, Brignole C. Pastorino F, Calarco E et al. Recent advances in the developmental origin of neuroblastoma: An overview. J Exp Clin Cancer Res. 2022;41:1-28.
3. Delahunty I, Nishiguchi G, Zhang Y, Fish A, Mayasundari A, Qi J et al. Investigating molecular glues as a new therapeutic approach to high-risk neuroblastoma. Cancer Res. 2023;83:481-81.
4. Cirmi S, Ferlazzo N, Gugliandolo A, Musumeci L, Mazzon E, Bramanti A et al. Moringin from Moringa oleifera seeds inhibits growth, arrests cell-cycle, and induces apoptosis of SH-SY5Y human neuroblastoma cells through the modulation of NF-ϰB and apoptotic related factors. Int J Mol Sci. 2019;20:1930.
5. Servi H, Keskin BE, Çelik S, Budak Ü, Kırmızıtaş FC, Bektaş EB. Essential oil composition and fatty acid profile of two endemic Gypsophila species from Turkey. Am J Essent Oil Nat Prod. 2017;5:16-20.
6. Altay A, Tohma H, Durmaz L, Taslimi P, Korkmaz M, Gulcin I et al. Preliminary phytochemical analysis and evaluation of in vitro antioxidant, antiproliferative, antidiabetic, and anticholinergics effects of endemic Gypsophila taxa from Turkey. J Food Biochem. 2019;43:e12908.
7. Zhang W, Luo JG, Zhang C, Kong LY. Different apoptotic effects of triterpenoid saponin-rich Gypsophila oldhamiana root extract on human hepatoma SMMC-7721 and normal human hepatic L02 cells. Biol Pharm Bull. 2013;36:1080-87.
8. Arslan I, Celik A, Melzig MF. Nebulosides A–B, novel triterpene saponins from under-ground parts of Gypsophila arrostii Guss. var. nebulosa. Bioorg Med Chem. 2013;21:1279-83.
9. Korkmaz M, Özçelik H. Economic importance of Gypsophila L., Ankyropetalum fenzl and Saponaria L.(Caryophyllaceae) taxa of Turkey. Afr J Biotechnol. 2011;10:9533-41.
10. Yücekutlu AN, Bildacı I. Determination of plant saponins and some of Gypsophila species: A review of the literature. Hacettepe J Biol Chem. 2008;36:129-35.
11. Li Y, Sun Y, Fan L, Zhang F, Meng J, Han J et al. Paris saponin VII inhibits growth of colorectal cancer cells through Ras signaling pathway. Biochem Pharmacol. 2014;88:150-7.
12. Chen PS, Shih YW, Huang HC, Cheng HW. Diosgenin, a steroidal saponin, inhibits migration and invasion of human prostate cancer PC-3 cells by reducing matrix metalloproteinases expression. PloS One 2011;6:e20164.
13. Lin X, Wei J, Chen Y, He P, Lin J, Tan S et al. Isoorientin from Gypsophila elegans induces apoptosis in liver cancer cells via mitochondrial-mediated pathway. J Ethnopharmacol. 2016;187:187-94.
14. Rad AS, Esmaeili S, Motamed SM, Hamzeloo-Moghadam M. Cytotoxicity of two Gypsophila species to human breast adenocarcinoma (MCF-7). Int J Acta. 2018;1:82-82.
15. Tian G, Zhou L, Zhong Y, Xu W, Bai H, Liu L et al. Experimental studies of the therapeutic effect of Gypsophila oldhamiana gypsogenin on Lewis lung cancer in mice. Chin Clin Oncol. 2008;5:206-10.
16. Liu Y, Li X, Jiang S, Ge Q. Inhibitory effect of Gypsophila oldhamiana gypsogenin on NCI-N87 gastric cancer cell line. Eur J Inflamm. 2018;16:2058739218818958.