Research Article

Yagmur Azbazdar, Selin Helvacioglu and Gunes Ozhan*

Gypsophila eriocalyx roots inhibit proliferation, migration, and TGF-β signaling in melanoma cells

https://doi.org/10.1515/tjb-2024-0193
Received August 8, 2024; accepted November 19, 2024;
published online January 8, 2025

Abstract

Objectives: Melanoma is a highly malignant and serious form of skin cancer. In addition to the standard treatments, complementary approaches, including phytotherapy, are also used to alleviate symptoms and improve patient well-being. This study aims to investigate the anticancer effects of *Gypsophila eriocalyx* (GE), an endemic species from Türkiye, on melanoma cells. We set out to determine the efficacy of GE in inhibiting melanoma cell proliferation, migration, and growth, and to explore its underlying mechanisms.

Methods: We examined the impact of GE on the proliferation of two melanoma cell lines, Malme-3M and SK-MEL-28, and assessed its developmental toxicity in zebrafish embryos. Next, we evaluated GE's influence on colony formation and wound healing in melanoma cells, as well as its ability to induce apoptosis and affect the TGF-β/Smad signaling pathway, by measuring pathway reporter activity and target gene expression.

Results: GE inhibited cell proliferation in melanoma cell lines at concentrations 104 to 488 times lower than those required for normal non-malignant L929 fibroblast cells. In zebrafish embryos, GE demonstrated developmental toxicity only at concentrations above 50 µg/mL. GE treatment significantly impaired the colony formation and wound healing abilities of melanoma cells, indicating reduced proliferation and migration. Moreover, GE induced apoptosis in melanoma cells and inhibited the TGF-β/Smad signaling pathway, as evidenced by decreased pathway reporter activity and target gene expression.

Conclusions: This study highlights the potential of GE as a novel therapeutic agent in melanoma treatment by demonstrating its ability to inhibit tumor growth and progression.

Keywords: *Gypsophila*; melanoma; proliferation; migration; apoptosis; TGF-β; zebrafish

Introduction

Skin cancer is the most prevalent type of cancer globally, primarily caused by excessive ultraviolet (UV) radiation exposure from the sun. Yet, a subset of individuals appears genetically predisposed to developing skin cancer [1]. Skin cancer is categorized into two main groups: melanoma and keratinocyte carcinoma, the latter of which includes basal cell carcinoma and squamous cell carcinoma. While melanoma represents only 1–3 % of skin cancer cases annually, it is responsible for approximately 65 % of skin cancer-related deaths due to its aggressive and metastatic nature [2]. Risk factors for melanoma include the number of melanocytic nevi, genetic predisposition, and a previous history of melanoma.

Despite significant advances in treatment, the prognosis for patients with advanced metastatic melanoma remains poor, with stage IV melanoma patients having a survival rate of less than one year. Current treatment options for melanoma include surgical removal for tumors larger than 2.0 mm and for primary melanomas that have metastasized. Surgery is often complemented by radiotherapy, immune checkpoint inhibitors, and targeted therapies, particularly for advanced melanomas with mutations in the B-Raf

***Corresponding author: Gunes Ozhan**, Izmir Biomedicine and Genome Center (IBG), Dokuz Eylul University Health Campus, Inciralti-Balcova, 35340, Izmir, Türkiye; and Department of Molecular Biology and Genetics, Izmir Institute of Technology, Urla, 35430, Izmir, Türkiye,
E-mail: gunesozhan@iyte.edu.tr. https://orcid.org/0000-0002-4806-5917
Yagmur Azbazdar, Izmir Biomedicine and Genome Center (IBG), Dokuz Eylul University Health Campus, Izmir, Türkiye; Izmir International Biomedicine and Genome Institute (IBG-Izmir), Dokuz Eylul University, Izmir, Türkiye; and Department of Biological Chemistry, University of California Los Angeles, Los Angeles, CA, USA. https://orcid.org/0000-0003-0806-1003
Selin Helvacioglu, Izmir Biomedicine and Genome Center (IBG), Dokuz Eylul University Health Campus, Izmir, Türkiye; and Department of Molecular Biology and Genetics, Izmir Institute of Technology, Izmir, Türkiye. https://orcid.org/0009-0002-8582-5257

proto-oncogene (BRAF) or c-KIT proto-oncogene [3, 4]. Tyrosine kinase inhibitors and checkpoint inhibitors have shown promising results in improving the prognosis and health outcomes of melanoma patients [5]. Additionally, genomic alterations in the mitogen-activated protein kinase (MAPK) and phosphoinositide-3-kinase (PI3K) pathways contribute to melanoma progression [6]. Consequently, inhibitors targeting KIT, BRAF, NRAS, and MAPK/ERK kinase (MEK) are frequently used in treatment [6, 7]. For metastases that cannot be surgically removed, chemotherapy is also an option, though it is generally less effective.

Given the limitations of conventional treatments and the potential benefits of natural compounds, there is increasing interest in complementary and alternative medicines (CAM) for melanoma treatment [8, 9]. Endemic medicinal plants are often explored for their biological and anticancer properties. *Gypsophila*, commonly known as baby's breath, is a genus within the Caryophyllaceae family and includes approximately 150 species [10]. Methanol extracts from various *Gypsophila* species have been analyzed for their phenolic and flavonoid content and tested for activities such as cytotoxicity, anti-cholinesterase, anti-tyrosinase, anti-amylase, and anti-glucosidase [11, 12]. This study focuses on the anticancer effects of *Gypsophila eriocalyx* (GE) root extracts, a species native to Türkiye, in two melanoma cell lines. Our results demonstrate that GE root extract exhibits greater toxicity towards Malme-3M and SK-MEL-28 melanoma cells compared to normal cells and zebrafish embryos. Furthermore, GE significantly inhibits melanoma cell growth and migration, induces apoptosis, and suppresses TGF-β signaling. These findings suggest that GE root extracts may offer a promising alternative or adjunctive therapy for melanoma treatment, warranting further investigation into their potential clinical applications.

Materials and methods

Preparation of root extracts from *G. eriocalyx*

G. eriocalyx (GE) was collected in June from Konya, Türkiye, and identified according to the Flora of Turkey [13, 14]. The roots were ground into a fine powder using a mortar and pestle. Two grams of the root powder were mixed with 100 mL of 80 % methanol in an Erlenmeyer flask and incubated at 60–65 °C for 6 h. The mixture was then filtered through filter paper to remove the solid residue. The resulting filtrate was transferred to sterile Petri dishes, and the methanol was evaporated under a fume hood. The dried extract was subsequently dissolved in dimethyl sulfoxide (DMSO) to achieve a final concentration of 100 mg/mL. The suspension was centrifuged to remove any remaining particulate matter, and the supernatant was used for all subsequent experiments.

Cell culture

L929 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with high glucose, supplemented with 10 % fetal bovine serum (FBS), at 37 °C in a 5 % CO_2 humidified incubator. Malme-3M and SK-MEL-28 cells were maintained in Roswell Park Memorial Institute (RPMI) 1,640 medium with 10 % FBS at 37 °C in a 5 % CO_2 humidified incubator.

MTT assay

Cells were seeded at a density of 1,000 cells per well in a 96-well plate, with a final volume of 100 µL per well. After incubation for 24 h at 37 °C, GE root extracts were added in serial dilutions: from 1,000 to 0.98 µg/mL for L929 cells, from 100 to 0.19 µg/mL for SK-MEL-28 cells, and from 400 to 0.39 µg/mL for Malme-3M cells. The cells were incubated for an additional 72 h. Subsequently, 15 µL of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL in PBS) was added to each well. After a 4-h incubation at 37 °C, the supernatant was discarded, and 100 µL of 0.5 % DMSO was added to dissolve the formazan crystals. The plate was wrapped in aluminum foil and shaken at 800 rpm for 20 min at room temperature. Absorbance was measured at 570 nm with a reference wavelength of 720 nm. The half-maximal inhibitory concentration (IC_{50}) of GE root extract was determined as 21.62 µg/mL for Malme-3M cells and 4.6 µg/mL for SK-MEL-28 cells. For subsequent experiments, GE root extracts were used at these IC_{50} concentrations. Control groups were treated with DMSO at the same concentrations used in GE root extract treatments.

Zebrafish embryo toxicity assay

Wild-type (wt) AB zebrafish (*Danio rerio*) embryos were dechorionated at 8 h post-fertilization (hpf) using pronase. Embryos were placed individually into the wells of a 96-well plate. GE root extracts were diluted in E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, 0.33 mM $MgSO_4$, 0.01 % methylene blue) and added to the wells in serial dilutions from 200 to 6.25 µg/mL. The control group received 0.2 % DMSO. Embryos and larvae were imaged at 32 and 56 hpf.

Colony formation assay

Cells were seeded at densities of 1,000 and 2,000 cells per well in a 6-well plate and allowed to form colonies for 6 days. GE root extracts were added on the second day of incubation. The medium containing GE extracts was refreshed every two days during the 6-day incubation period. After 6 days at 37 °C, the supernatant was removed, and the cells were fixed with 100 % methanol for 10 min at −20 °C. Cells were then stained with crystal violet (0.5 % w/v in distilled water) for 20 min at room temperature. After staining, cells were washed three times with distilled water, allowed to air dry, and imaged under a light microscope.

Wound healing assay

Malme-3M and SK-MEL-28 cells were seeded in 6-well plates. On the second day, a wound was created by scratching the cell monolayer with a 200 µL pipette tip. The cells were washed with PBS to remove debris and then incubated with GE root extracts in 1 mL of RPMI 1640 medium containing reduced serum (2 % FBS) at 37 °C for 24 h. The wound area was measured and expressed as a percentage relative to the control.

Apoptosis assay

Malme-3M and SK-MEL-28 cells were seeded in 6-well plates and treated with GE root extract for 24 h at 37 °C. For the positive control, cells were exposed to 0.3 mM H_2O_2 for 18 h. Supernatants and trypsinized cells were collected, washed with PBS, and stained using the FITC Annexin V Apoptosis Detection Kit with 7-AAD (BioLegend, San Diego, CA, USA) according to the manufacturer's protocol [15]. Flow cytometry was performed using a BD Fortessa, and data were analyzed with FlowJo 8.8.6 software (Tree Star Inc, Ashland, OR, USA). Experiments were conducted in triplicate, and data are representative of three independent experiments.

Transfection and luciferase assay

Cells were seeded in triplicate in 24-well plates. For the TGF-β reporter assay, cells were transfected with 200 ng of the firefly luciferase reporter plasmid pSBE4-Luc (Addgene, MA, USA), which contains four copies of the Smad binding site, and 30 ng of the renilla luciferase reporter plasmid pRL-TK (Promega, WI, USA), using FuGENE HD transfection reagent (1 µg DNA/1 µL FuGENE) (Promega, WI, USA). After incubation at 37 °C for 4 h, GE root extracts were added to the cells and incubated for another 24 h. Reporter activity was measured using the Dual-Luciferase Reporter Assay System (Promega, WI, USA).

Quantitative PCR (qPCR)

RNA was isolated from cells using the RNeasy Mini Kit (Qiagen, Hilden, Germany). cDNA was synthesized from RNA using iScript Reverse Transcriptase (RT) (Bio-Rad, CA, USA) with a 1:1 mixture of random and oligo (dT) primers, following the manufacturer's instructions. RT was replaced with water in the −RT controls. qPCR was performed in triplicate using 1:100 diluted cDNA, primers, and GoTaq qPCR Master Mix (Promega, Madison, WI, USA) on an Applied Biosystems 7,500 Fast Real-Time PCR System (Foster City, CA, USA). The qPCR cycle conditions were as follows: an initial holding stage at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s. The melt curve stage consisted of 95 °C for 15 s, 60 °C for 60 s, 95 °C for 30 s, and a final step at 60 °C for 60 s. Human *RPL13A* was used as the reference gene for normalization. The following primers were used: human *RPL13A* forward 5′-CCGCCCTACGACAAGAAA-3′ and reverse 5′-CAGGGTGG-CTGTCACTGC-3′, human *SNAI2* forward 5′-GCTACCCAATGG-CCTCTCTC-3′ and reverse 5′-CTTCAATGGCATGGGGGTCT-3′, human *FOXP3* forward 5′-CAGCACATTCCCAGAGTTCCT-3′ and reverse 5′-TCATTGAGTGTCCGCTGCTT-3′, human *SOX4* forward 5′-GCACTAGGACGTCTGCCTTT-3′ and reverse 5′-ACACGGCATATTGCACAGGA-3′. The product sizes for each primer set are as follows: *RPL13A*, 141 bp; *SNAI2*, 170 bp; *FOXP3*, 132 bp; and *SOX4*, 92 bp. Data were presented as the mean ± SD (standard deviation) of three samples. Analysis was performed using GraphPad Prism 8 software (GraphPad Software Inc., San Diego, CA, USA).

Results

Effect of GE root extract on human melanoma cell viability

To examine whether *G. eriocalyx* (GE) root extract influenced cell viability in melanoma cells, we treated two different types of human malignant melanoma cell lines, Malme-3M and SK-MEL-28, with GE root extract at the concentrations of 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 and 0.39 µg/mL; and 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 and 0.19 µg/mL, respectively. GE reduced cell viability in a dose-dependent manner in both melanoma cell lines as detected by the MTT assay (Figure 1A and B). The IC_{50} values were 21.62 and 4.6 µg/mL for Malme-3M and SK-MEL-28 cells, respectively, and were used in subsequent experiments. Additionally, we measured the viability of

L929 cells, non-malignant fibroblast cells, upon exposure to different concentrations of GE root extract. The IC_{50} value was 2,246 µg/mL (Figure 1C). Notably, concentrations up to 1,000 µg/mL were tested, with cell viability remaining around 75 %. Therefore, the IC_{50} value was theoretically estimated. These results indicate that GE root extract is approximately 104 and 488 times more toxic to Malme-3M and SK-MEL-28 cells, respectively, than to L929 cells. To determine the developmental toxicity of GE *in vivo*, we took advantage of the zebrafish embryos/larvae. Control embryos developed normally, while low concentrations of GE (12.5–25 µg/mL) caused no visible abnormalities (Figure S1). At 50 µg/mL, developmental delay was evident by 32 hpf, with complete destruction of the embryos by 56 hpf. Higher concentrations (100–200 µg/mL) caused severe developmental disruption or death at both time points, with embryos appearing as undifferentiated cell masses. This indicates that GE is highly toxic at concentrations above 50 µg/mL, particularly with prolonged exposure (Figure 1D). In the groups treated with 100, 50, 25, 12.5, and 6.25 µg/mL of GE, the percentage of live embryos at 24 hpf was 27 , 59, 65, 86, and 84 %, respectively. Thus, GE appears to become more toxic only above 50 µg/mL *in vivo*.

GE inhibits colony formation and wound healing in melanoma cell lines

To reveal the influence of GE root extracts on melanoma cell proliferation, we performed a colony formation assay to measure the ability of a single cell to grow in a colony. While colonization was evident in the control groups, GE dramatically reduced the colony-forming ability of both Malme-3M and SK-MEL-28 cells (Figure 2A and B). Next, to assess whether GE affects cell migration and proliferation, we used a scratch assay, which enables cell tracking during the closure of a wound. 24 h after the wound application, the closure in both cell lines was slower in the GE-treated experimental group than in the DMSO-treated control group (Figure 2C and D). The percentage of recovery in Malme-3M cells was 53 and 13 % in the control and GE groups, respectively (Figure 2C). The recovery rate in SK-MEL-28 cells was 72 and 25 % for the corresponding groups (Figure 2D). Taken together, these results indicate that GE efficiently suppresses the proliferation and migration of melanoma cells.

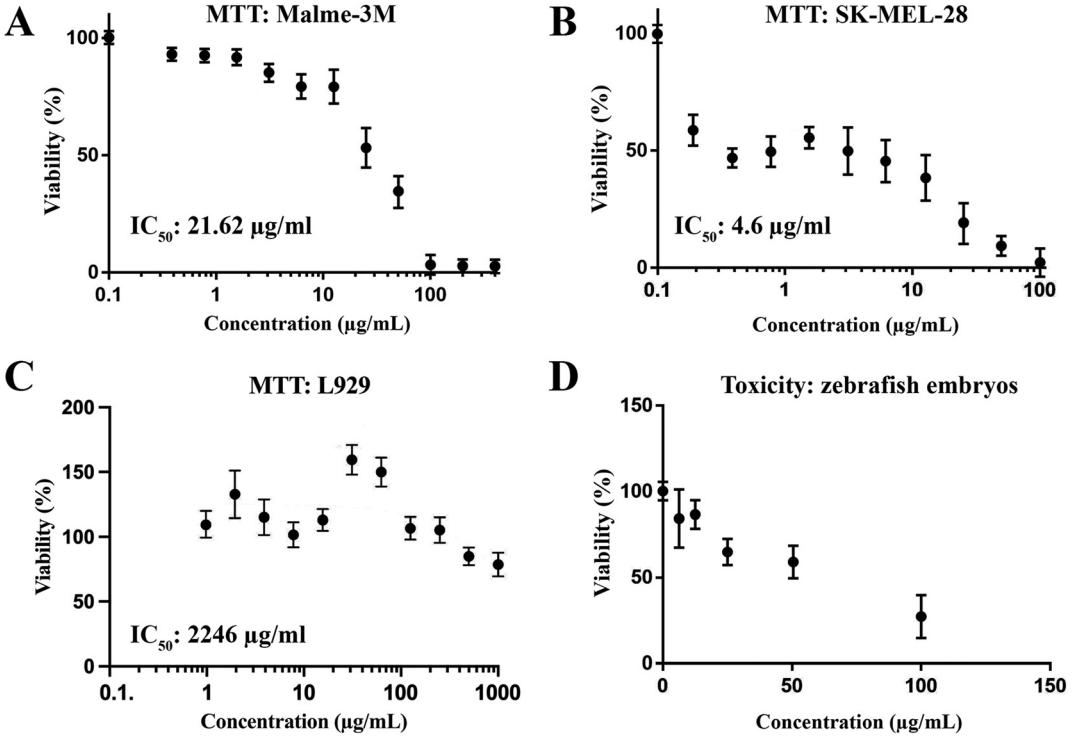

Figure 1: Effect of GE root extract on human melanoma cell viability. (A–B) MTT analysis demonstrating IC_{50} values for Malme-3M (21.62 µg/mL) and SK-MEL-28 (4.6 µg/mL) cells, respectively. (C) IC_{50} value for L929 cells (2,246 µg/mL). (D) Toxicity analysis on zebrafish embryos treated with GE root extract. Absorbance was measured at 570 nm with a reference wavelength of 720 nm. Data are presented as the mean ± standard deviation (SD) from three independent replicates.

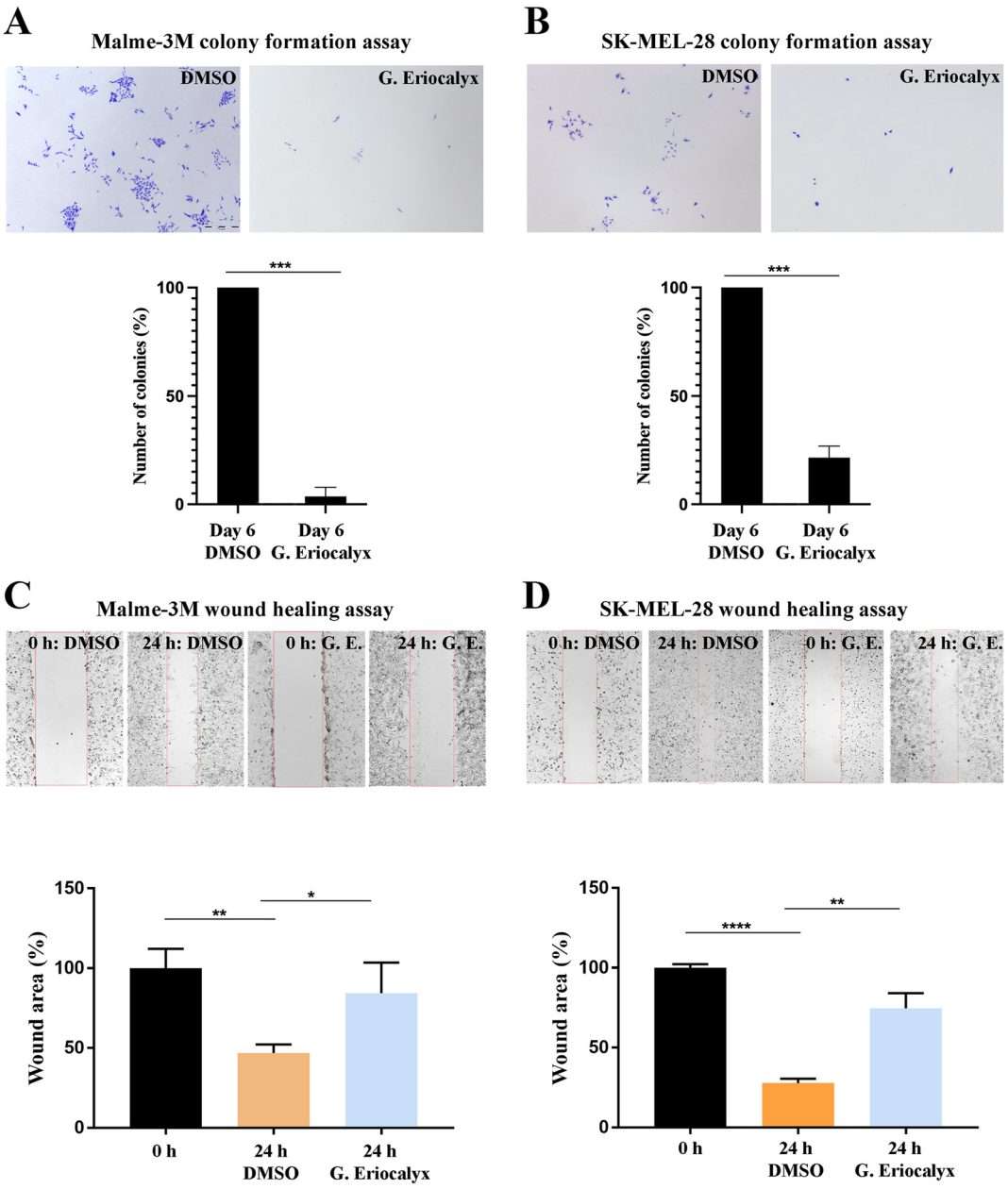

Figure 2: GE inhibits colony formation and wound healing in melanoma cell lines. (A–B) Colony formation assay for Malme-3M and SK-MEL-28 cells treated with DMSO (control) and GE root extract. Statistical significance was evaluated using the Mann–Whitney U test. ***p<0.001. Error bars represent SD. Six independent experiments were performed. (C–D) Wound healing assay for Malme-3M and SK-MEL-28 cells treated with DMSO (control) and GE root extract. Dashed red lines indicate the area measured, highlighting the gap created by the scratch and used for assessing cell migration over time. Statistical significance was evaluated using an unpaired *t*-test. ****p<0.0001, **p<0.01, and *p<0.05. Error bars represent SD. Three independent experiments were performed.

GE enhances apoptosis in melanoma cells

Since the loss of apoptotic control allows cancer cells to survive longer, activation of apoptosis is a critical mechanism for controlling cancer cells. To determine whether GE can induce apoptosis in melanoma cells, we treated Malme-3M and SK-MEL-28 cells with the GE root extract and quantified the cells at the early and late stages of apoptosis using flow cytometry. The positive control, H_2O_2, resulted in the death of almost all cells (Figure 3A–D). GE treatment induced apoptosis in Malme-3M (Figure 3A and C; early apoptotic cells 65.5 % and late apoptotic cells 1.97 %) and SK-MEL-28 (Figure 3B and D; early apoptotic cells 77.8 % and late apoptotic cells 18.1 %) cell lines. Strikingly, the percentage of

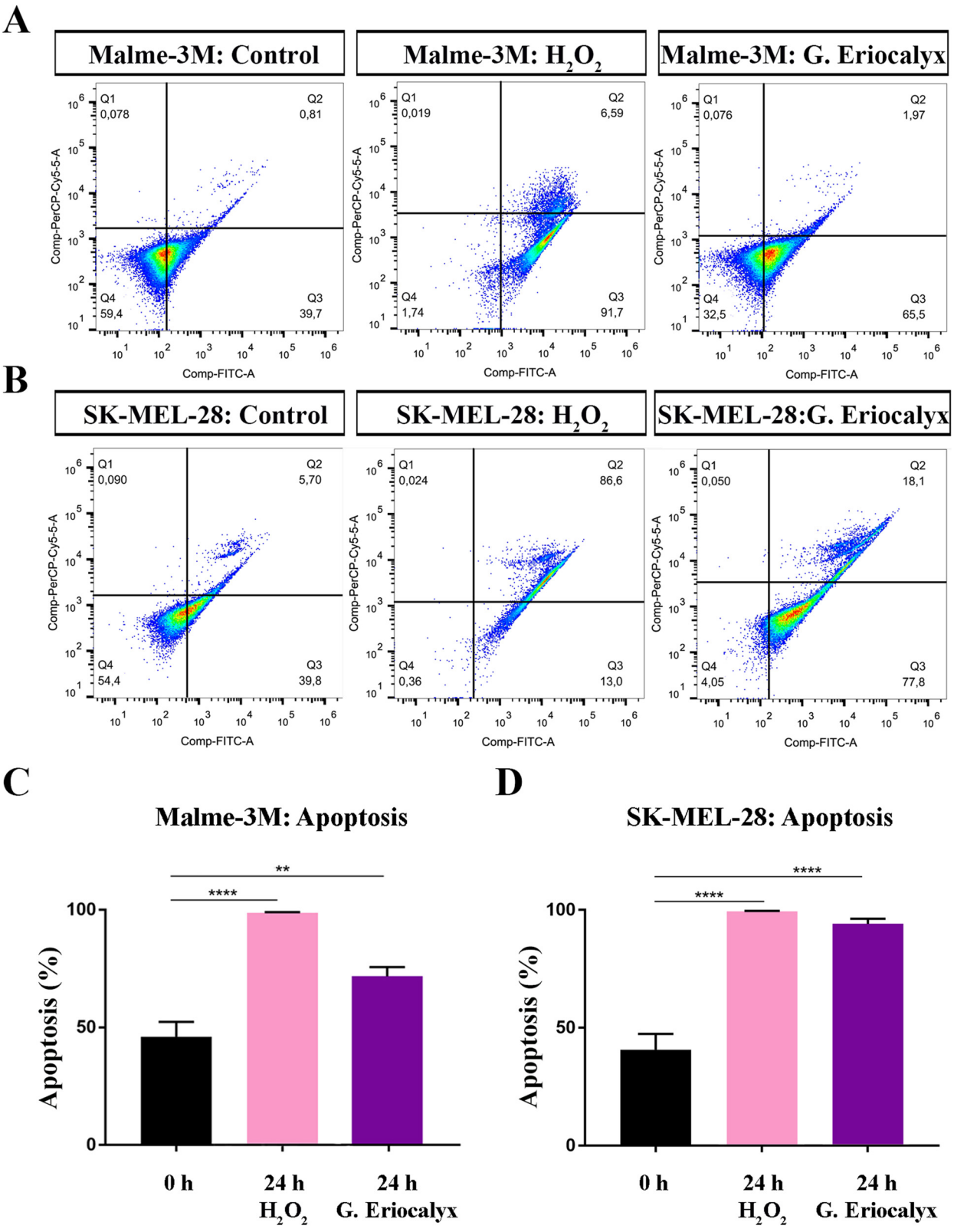

Figure 3: GE enhances apoptosis in melanoma cells. (A–B) Apoptosis assay on Malme-3M and SK-MEL-28 cells using flow cytometry after staining with annexin V-FITC and 7AAD. Representative scatter plots of 7AAD (y-axis) vs. Annexin V-FITC (x-axis). (C–D) Percentage of apoptotic cells in Malme-3M and (D) SK-MEL-28 cells. H_2O_2 was used as a positive control. Statistical significance was evaluated using an unpaired *t*-test. ****p<0.0001 and **p<0.01. Error bars represent SD. All experiments were performed in three replicates. Three independent experiments were conducted.

necrotic cells was substantially low upon GE exposure. Thus, GE root extract appears to act as a potent apoptosis inducer in melanoma cells.

GE inhibits TGF-β signaling in melanoma cells

The transforming growth factor-β (TGF-β) signaling pathway has been reported to be activated in a cell-autonomous manner in various melanoma cell lines and has attracted considerable attention as a potential therapeutic target [16]. Thus, we investigated whether GE influences TGF-β signaling. According to the activation of the pSBE4-Luc, a reporter of the TGF-β/Smad signaling pathway, GE treatment significantly suppressed TGF-β signaling in Malme-3M and SK-MEL-28 cells (Figure 4A and B). To further support these data, we performed qPCR and found that the expression of key transcriptional targets of the TGF-β signaling pathway was notably reduced in the melanoma cell lines (Figure 4C and D). These results indicate that GE root extracts inhibit endogenously activated TGF-β signaling in Malme-3M and SK-MEL-28 cells.

Discussion

Medicinal plants have long been exploited for the treatment of cancer, due to their non-toxic effects on normal cells and their cytotoxicity on cancer cells. The anti-cancer effects of these plants in metastatic melanoma have also attracted attention. In this study, we examined the influence of *G. eriocalyx* (GE) species on melanoma cells concerning several features of cancer cells.

Depending on the stage and location of the melanoma, surgery, immunotherapy, targeted therapy, chemotherapy, photodynamic therapy, and radiation therapy have been

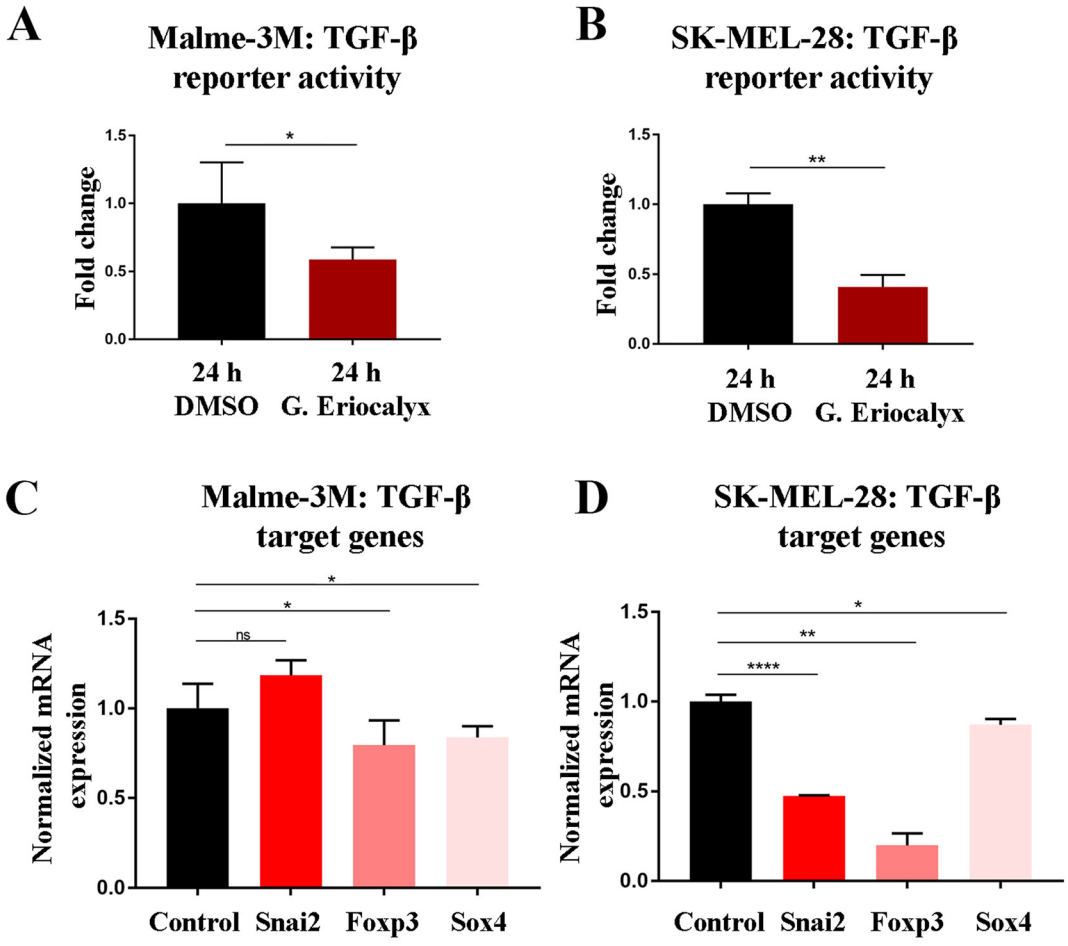

Figure 4: GE inhibits TGF-β signaling in melanoma cells. (A–B) Average and SD of the mean (error bars) values of luciferase reporter activity monitoring TGF-β signaling activity (normalized to renilla luciferase activity) in Malme-3M and SK-MEL-28 cells. (C–D) Expression levels of target genes of the TGF-β signaling pathway determined by qPCR in Malme-3M and SK-MEL-28 cells. Statistical significance was evaluated using an unpaired *t*-test. ****p<0.0001, **p<0.01, *p<0.05 and ns non-significant. Error bars represent SD (n=3). Three independent experiments were conducted.

used individually or in combination for melanoma treatment [3]. Nevertheless, each of these strategies has its drawbacks, including low efficacy, acquired resistance, and severe side effects. At this point, natural compounds have been widely investigated for their anticancer effects in the treatment of melanoma [8, 17, 18]. For example, the plant flavonol quercetin has been found to reduce cell viability and induce apoptosis in melanoma cells in a concentration-dependent manner [19, 20]. Kaempferol, epigallocatechin-3-gallate, amentoflavone, β-Carotene, fucoxanthin, vitamin E, and resveratrol are among the phytochemicals and natural compounds with growth-inhibiting, apoptosis-inducing, and chemotherapy drug-sensitizing roles in melanoma [21–24]. Crude plant extracts from *Hypericum perforatum, Withania somnifera*, *Viscum album, Calendula officinalis, Aloe vera, Alpinia oxyphylla, Salvia* species, *Rumex* species, and various other species have also been discovered to manifest anti-melanoma effects *in vitro* and *in vivo* [25–29]. Nevertheless, it should be noted that these compounds might have opposite or adverse effects on cancer cells depending on the concentrations used and need to be handled with caution [20, 30, 31]. The lack of regulation in the field results in a lack of knowledge of minimum effective and maximum safe concentrations as well as bioactivity of the compounds, necessitating standardization of formulations.

Several studies have revealed that compounds isolated from species of *Gypsophila* genus have inhibitory effects on different processes occurring in cancer cells. For example, saponins from different *Gypsophila* species have been shown to exhibit cytotoxicity in lymphoma and monocyte/macrophage cell lines [32, 33]. Likewise, oldhamianoside II, a triterpenoid saponin isolated from the roots of *G. Oldhamiana*, exerts anticancer activity by inhibiting migration and reversing EMT in prostate cancer cell lines [34]. Oldhamianoside II has also been shown to upregulate Wnt antagonist expression and thus inhibit Wnt/β-catenin signaling. Our unpublished data have shown a parallel inhibition of Wnt/β-catenin signaling in response to GE treatment in melanoma cells, suggesting a potential anticancer role of *Gypsophila* species through inhibition of Wnt/β-catenin signaling.

Complementary studies on the natural flavonoid isoorientin, isolated from *Gypsophila elegans*, have shown that isoorientin reduced the viability and migration of HepG2 hepatocellular carcinoma (HCC) cells and hepatic stellate cells while inducing apoptosis in both cells [35, 36]. Moreover, four endemic *Gysophila* species of Türkiye, *Gypsophila pallida*, *Gypsophila arrosti*, *Gypsophila tuberculosa,* and *G. eriocalyx*, which have been reported to be rich in phenolics and flavonoids, exerted antiproliferative activities on HCC, colorectal carcinoma, and breast cancer cell lines [37]. Among the four species, *G. eriocalyx* had the highest antiproliferative effect and the lowest IC_{50} values in all three cancer cell lines. Interestingly, while the proliferation-promoting and apoptosis-inhibiting roles of *G. eriocalyx* show a parallelism between melanoma cells and other cancer cells, the IC_{50} values we obtained in melanoma cells were much lower than in those three cancer cells. A possible explanation for this difference is that our extraction method generates a higher yield of bioactive compounds from GE roots and, correspondingly, a more potent anticancer activity.

TGF-β signaling functions as a tumor suppressor in normal cells and early-stage cancer but often shifts to promoting tumor progression in later stages [38–40]. TGF-β signaling has been associated with cancer progression through the induction of epithelial-mesenchymal transition (EMT), promotion of cell migration, invasion, metastasis and an immunosuppressive tumor microenvironment [41]. The overexpression or dysregulation of TGF-β signaling components has been reported in melanoma, making it a compelling target for further investigation [38, 42, 43]. In melanoma, elevated TGF-β activity is common and known to contribute to melanoma progression [44]. GE-derived isoorientin has been found to inhibit the TGFβ1/Smad signaling pathway and prevent chemical-induced liver fibrosis in a rat model of fibrosis [45]. Our results support this finding by revealing that GE root extracts significantly suppress TGFβ signaling at the transcriptional level. Thus, understanding how TGF-β signaling pathway contributes to melanoma progression could offer insights into potential therapeutic strategies.

A recent study on GE highlights the biological properties of its methanol, ethanol, and water extracts, emphasizing their antioxidant, antimicrobial, and anticancer activities [46]. Our comparison of IC_{50} values shows that GE root extract is more potent in melanoma cells (Malme-3M and SK-MEL-28) than the water, methanol, and ethanol extracts in breast cancer cells. The significantly lower IC_{50} values for melanoma cells suggest a stronger cytotoxic effect, indicating GE's potential as an effective melanoma treatment. These findings support GE's consistent anticancer effects across different cancer types, underscoring its versatility as a therapeutic agent. Further studies are needed to explore the underlying reasons for these differences.

The influence of GE root extract on melanoma cells underscores its potential as a therapeutic agent. The significant reduction in cell viability and colony formation, along with the suppression of wound healing capabilities, strongly suggests that GE impedes melanoma cell proliferation and migration. The observed induction of apoptosis, particularly the low percentage of necrotic cells, highlights GE's potential specificity in promoting programmed cell death over necrosis, which is advantageous for reducing unwanted tissue

damage. Moreover, the inhibition of TGF-β signaling, a pathway known to contribute to melanoma progression and metastasis, indicates a possible mechanism through which GE exerts its anti-cancer effects. Collectively, these findings suggest that GE root extract may serve as a multi-faceted anti-melanoma agent, warranting further investigation into its molecular targets and the development of optimized formulations for clinical applications. Additionally, the distinct differential toxicity of GE between malignant and non-malignant cells emphasizes its therapeutic promise with a potentially favorable safety profile. Future studies should focus on elucidating the precise bioactive compounds within GE, their individual and synergistic effects, and their *in vivo* efficacy and safety in animal models.

Conclusions

There is very little information in the literature about *G. eriocalyx*, an endemic plant native to Türkiye. In the only previous study with this species, the antiproliferative activity of *G. eriocalyx* has been displayed in liver, colon, and breast cancer cell lines. Thus, this is the first report on the cytotoxic effect and anticancer activity of GE root extract on melanoma cells. Moreover, we reveal that this anti-melanoma activity could act through suppression of the TGFβ1/Smad signaling pathway. Future work on melanoma and other cancers will help understand the underlying molecular mechanisms of this anticancer mechanism.

Acknowledgments: We thank Zeynep Önder and Utku Bora Döke for their help in the preparation of the *Gypsophila Eriocalyx* root extracts, and Izmir Biomedicine and Genome Center Flow Cytometry and Cell Sorting Core Facility for providing help in the flow cytometry assay.
Research ethics: Not applicable.
Informed consent: Not applicable.
Author contributions: The authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Use of Large Language Models, AI and Machine learning Tools: None declared.
Conflict of interest: The authors state no conflict of interest.
Research funding: This work has been supported by the European Molecular Biology Organization (EMBO) Installation Grant (IG 3024). YA was supported by the TUBITAK 2211-C National Priority Areas Doctoral Scholarship Program and the Council of Higher Education (YÖK) 100/2000 Ph.D. Scholarship Program. SH was supported by the TUBITAK 2210-A National MSc Scholarship Program.
Data availability: The raw data can be obtained on request from the corresponding author.

References

1. Esteva A, Kuprel B, Novoa RA, Ko J, Swetter SM, Blau HM, et al. Dermatologist-level classification of skin cancer with deep neural networks. Nature 2017;542:115–8.
2. Pavri SN, Clune J, Ariyan S, Narayan D. Malignant melanoma: beyond the basics. Plast Reconstr Surg 2016;138:330e–40e.
3. Domingues B, Lopes JM, Soares P, Pópulo H. Melanoma treatment in review. ImmunoTargets Ther 2018;7:35–49.
4. Rager T, Eckburg A, Patel M, Qiu R, Gantiwala S, Dovalovsky K, et al. Treatment of metastatic melanoma with a combination of immunotherapies and molecularly targeted therapies. Cancers 2022; 14. https://doi.org/10.3390/cancers14153779.
5. McKean M, Oba J, Ma J, Roth KG, Wang WL, Macedo MP, et al. Tyrosine kinase inhibitor and immune checkpoint inhibitor responses in KIT-mutant metastatic melanoma. J Invest Dermatol 2019;139:728–31.
6. Leonardi GC, Falzone L, Salemi R, Zanghì A, Spandidos DA, McCubrey JA, et al. Cutaneous melanoma: from pathogenesis to therapy (Review). Int J Oncol 2018;52:1071–80.
7. Schadendorf D, van Akkooi ACJ, Berking C, Griewank KG, Gutzmer R, Hauschild A, et al. Melanoma. Lancet 2018;392:971–84.
8. Chinembiri TN, Du Plessis LH, Gerber M, Hamman JH, Du Plessis J. Review of natural compounds for potential skin cancer treatment. Molecules 2014;19:11679–721.
9. Clairet AL, Boiteux-Jurain M, Curtit E, Jeannin M, Gérard B, Nerich V, et al. Interaction between phytotherapy and oral anticancer agents: prospective study and literature review. Med Oncol 2019;36:45.
10. Royal Botanic Gardens, Kew. [Internet] Available from: https://powo.science.kew.org/.
11. Zheleva-Dimitrova D, Zengin G, Balabanova V, Voynikov Y, Lozanov V, Lazarova I, et al. Chemical characterization with in vitro biological activities of *Gypsophila* species. J Pharmaceut Biomed Anal 2018;155: 56–69.
12. Pertuit D, Avunduk S, Mitaine-Offer A-C, Miyamoto T, Tanaka C, Paululat T, et al. Triterpenoid saponins from the roots of two *Gypsophila* species. Phytochemistry 2014;102:182–8.
13. Davis PH. Flora of Turkey and the East Aegean Islands. Edinburgh, Scotland: Edinburgh University Press; 1965.
14. [Internet]. Available from: https://turkiyebitkileri.com/en/.
15. Ozalp O, Cark O, Azbazdar Y, Haykir B, Cucun G, Kucukaylak I, et al. Nradd acts as a negative feedback regulator of wnt/β-catenin signaling and promotes apoptosis. Biomolecules 2021;11. https://doi.org/10.3390/biom11010100.
16. Busse A, Keilholz U. Role of TGF-β in melanoma. Curr Pharmaceut Biotechnol 2011;12:2165–75.
17. Albuquerque KRS, Pacheco NM, del Rosario Loyo Casao T, de Melo FCSA, Novaes RD, Gonçalves RV. Applicability of plant extracts in preclinical studies of melanoma: a systematic review. Mediat Inflamm 2018;2018:6797924.
18. El-Harakeh M, Al-Ghadban S, Safi R. Medicinal plants towards modeling skin cancer. Curr Drug Targets 2021;22:148–61.
19. Cao H-H, Tse AK-W, Kwan H-Y, Yu H, Cheng C-Y, Su T, et al. Quercetin exerts anti-melanoma activities and inhibits STAT3 signaling. Biochem Pharmacol 2014;87:424–34.

20. Olson ER, Melton T, Dickinson SE, Dong Z, Alberts DS, Bowden GT. Quercetin potentiates UVB-Induced c-Fos expression: implications for its use as a chemopreventive agent. Cancer Prev Res 2010;3:876–84.
21. Guruvayoorappan C, Kuttan G. Amentoflavone inhibits experimental tumor metastasis through a regulatory mechanism involving MMP-2, MMP-9, prolyl hydroxylase, lysyl oxidase, VEGF, ERK-1, ERK-2, STAT-1, nm23 and cytokines in lung tissues of C57bl/6 mice. Immunopharmacol Immunotoxicol 2008;30:711–27.
22. Nihal M, Roelke CT, Wood GS. Anti-melanoma effects of vorinostat in combination with polyphenolic antioxidant (−)-Epigallocatechin-3-Gallate (EGCG). Pharmaceut Res 2010;27:1103–14.
23. Kim K-N, Ahn G, Heo S-J, Kang S-M, Kang M-C, Yang H-M, et al. Inhibition of tumor growth *in vitro* and *in vivo* by fucoxanthin against melanoma B16F10 cells. Environ Toxicol Pharmacol 2013;35:39–46.
24. Gatouillat G, Balasse E, Joseph-Pietras D, Morjani H, Madoulet C. Resveratrol induces cell-cycle disruption and apoptosis in chemoresistant B16 melanoma. J Cell Biochem 2010;110:893–902.
25. Chandu A, Kumar S, Bhattacharjee C, Debnath S. Cytotoxicity study of plant *Aloe vera* (Linn). Chronicles Young Sci 2012;3:233.
26. Jiménez-Medina E, Garcia-Lora A, Paco L, Algarra I, Collado A, Garrido F. A new extract of the plant calendula officinalis produces a dual *in vitro* effect: cytotoxic anti-tumor activity and lymphocyte activation. BMC Cancer 2006;6:119.
27. Lee C-C, Chiu C-C, Liao W-T, Wu P-F, Chen Y-T, Huang K-C, et al. *Alpinia oxyphylla* Miq. bioactive extracts from supercritical fluid carbon dioxide extraction. Biochem Eng J 2013;78:101–7.
28. Menichini G, Alfano C, Marrelli M, Toniolo C, Provenzano E, Statti GA, et al. *Hypericum perforatum* L. subsp. perforatum induces inhibition of free radicals and enhanced phototoxicity in human melanoma cells under ultraviolet light. Cell Prolif 2013;46:193–202.
29. Aydoğmuş-Öztürk F, Jahan H, Öztürk M, Günaydın K, Choudhary MI. Preclinical study of the medicinal plants for the treatment of malignant melanoma. Mol Biol Rep 2020;47:5975–83.
30. Philips N, Keller T, Holmes C. Reciprocal effects of ascorbate on cancer cell growth and the expression of matrix metalloproteinases and transforming growth factor-β. Cancer letters 2007;256:49–55.
31. Rosner K, Röpke C, Pless V, Skovgaard GL. Late type apoptosis and apoptosis free lethal effect of quercetin in human melanoma. Biosci, Biotechnol, Biochem 2006;70:2169–77.
32. Gevrenova R, Joubert O, Mandova T, Zaiou M, Chapleur Y, Henry M. Cytotoxic effects of four *Caryophyllaceae* species extracts on macrophage cell lines. Pharmaceut Biol 2014;52:919–25.
33. Gevrenova R, Zaharieva MM, Kroumov AD, Voutquenne-Nazabadioko L, Zheleva-Dimitrova D, Balabanova V, et al. Gypsophila saponins enhance the cytotoxicity of etoposide in HD-MY-Z lymphoma cells. Food Chem Toxicol 2019;133:110777.
34. Li K, Zhan X, Sun J, Wang T, Dong H, Jing F, et al. Oldhamianoside II inhibits prostate cancer progression via regulation of EMT and the Wnt/β-catenin signaling pathway. Oncol Lett 2018;15:9457–63.
35. 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.
36. Bai F, Huang Q, Wei J, Lv S, Chen Y, Liang C, et al. *Gypsophila elegans* isoorientin-2″-O-α-l-arabinopyranosyl ameliorates porcine serum-induced immune liver fibrosis by inhibiting NF-κB signaling pathway and suppressing HSC activation. Int Immunopharm 2018;54:60–7.
37. 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.
38. Katkat E, Demirci Y, Heger G, Karagulle D, Papatheodorou I, Brazma A, et al. Canonical Wnt and TGF-β/BMP signaling enhance melanocyte regeneration but suppress invasiveness, migration, and proliferation of melanoma cells. Front Cell Dev Biol 2023;11:1297910.
39. Giarratana AO, Prendergast CM, Salvatore MM, Capaccione KM. TGF-β signaling: critical nexus of fibrogenesis and cancer. J Transl Med 2024; 22:594.
40. Lebrun JJ. The dual role of TGFβ in human cancer: from tumor suppression to cancer metastasis. ISRN Mol Biol 2012;2012:381428.
41. Batlle E, Massagué J. Transforming growth factor-β signaling in immunity and cancer. Immunity 2019;50:924–40.
42. Javelaud D, Alexaki VI, Mauviel A. Transforming growth factor-beta in cutaneous melanoma. Pigm Cell Melanoma Res 2008;21:123–32.
43. Lauden L, Siewiera J, Boukouaci W, Ramgolam K, Mourah S, Lebbe C, et al. TGF-β-induced (TGFBI) protein in melanoma: a signature of high metastatic potential. J Invest Dermatol 2014;134:1675–85.
44. Kodama S, Podyma-Inoue KA, Uchihashi T, Kurioka K, Takahashi H, Sugauchi A, et al. Progression of melanoma is suppressed by targeting all transforming growth factor-β isoforms with an Fc chimeric receptor. Oncol Rep 2021;46:197.
45. Lin X, Chen Y, Lv S, Tan S, Zhang S, Huang R, et al. Gypsophila elegans isoorientin attenuates CCl_4-induced hepatic fibrosis in rats via modulation of NF-κB and TGF-β1/Smad signaling pathways. Int Immunopharm 2015;28:305–12.
46. İnanir M, Uçar E, Tüzün B, Eruygur N, Ataş M, Akpulat HA. The pharmacological properties of *Gypsophila eriocalyx*: the endemic medicinal plant of northern central Turkey. Int J Biol Macromol 2024; 266:130943.

Supplementary Material: This article contains supplementary material (https://doi.org/10.1515/tjb-2024-0193).