

Antiproliferative Effects of Galium Aparine in SNU-1 Gastric Cancer Cells and Its Association with Growth Signaling Pathways

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ABSTRACT


Galium aparine (G. aparine) is a plant with a long history of ethnopharmacological use and has been investigated for various biological activities, including potential anticancer effects. The present study aimed to investigate the in vitro effects of G. aparine extract on SNU-1 human gastric carcinoma cells and to examine its potential role in the inhibition of cell proliferation. Our experimental setup involved exposing SNU-1 cells to G. aparine (12.5–200 µg/mL) for 24 hours. Cell viability was then evaluated using the XTT assay, and ELISA measured the levels of growth signal factors mTOR, HER-2, EGFR, and ERK-1/2. The XTT assay revealed a significant reduction in cell viability ($p < 0.001$). Furthermore, ELISA measurements showed that G. aparine treatment notably decreased the levels of crucial growth signal factors, including mTOR, HER-2, EGFR, and ERK-1/2 ($p < 0.05$ to $p < 0.01$). These findings suggest that the antiproliferative activity of Galium aparine against gastric cancer cells may be associated with the modulation of key growth signaling pathways involved in cancer cell survival and proliferation. This highlights the potential of G. aparine as a natural agent that could be further investigated as a complementary or alternative approach for gastric cancer research.


Keywords: Anticancer, Galium aparine, Gastric carcinoma, Growth signaling pathways, SNU-1 cells.



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1. Introduction

Gastric cancer (GC) poses a significant global public health challenge. It ranks as the fifth most common cancer and stands as the third leading cause of cancer-related mortality worldwide [1]. Despite significant advances in surgical techniques and chemotherapeutic strategies, the prognosis for advanced-stage gastric cancer continues to be poor, with a 5-year survival rate of less than 45% [2]. The heterogeneity of gastric tumors, their late diagnosis, and the development of resistance to conventional therapies pose significant obstacles in improving patient outcomes [3]. These limitations underscore the urgent need to explore novel therapeutic agents, particularly those derived from natural sources, which may offer better safety profiles and unique mechanisms of action [4]. Galium aparine, commonly known as cleavers or goosegrass, is a climbing herbaceous plant from the Rubiaceae family. The Galium genus is well represented in Turkey, with around 101 species documented. In Anatolia, these plants are commonly known as 'Yogurt herb' due to their traditional use in curdling milk for cheese production. Across Europe and Asia, they have also been used in folk medicine for their diuretic, anti-inflammatory, and lymphatic system-supporting properties, as well as for treating fever, cancer, high blood pressure, gout, epilepsy,

and to promote wound healing [5–7]. Recent phytochemical analyses have revealed that G. aparine contains a range of biologically active compounds, including iridoids, flavonoids, and phenolic acids. These constituents are known to possess antioxidant, anti-inflammatory, and potentially anti-tumor properties, prompting a growing interest in the plant as a candidate for cancer research [8].

In the context of oncology, medicinal plants like G. aparine offer a promising avenue for drug discovery due to their structural diversity and ability to modulate various cellular pathways. Prior investigations have suggested that extracts from G. aparine can exhibit cytotoxic effects on certain cancer cell lines, though the mechanisms remain insufficiently understood [9]. However, no prior studies have specifically evaluated its effect on human gastric cancer cells, particularly the SNU1 cell line, which represents poorly differentiated gastric carcinoma and serves as a useful model for testing new therapeutic agents. We selected Galium aparine for this study due to its rich ethnopharmacological background and its underexplored potential in cancer treatment. Given its bioactive phytochemical content and preliminary indications of anticancer activity [10], this plant presents

a compelling candidate for further evaluation in gastric cancer models. To elucidate the anti-cancer effects of *G. aparine*, we investigated its cytotoxicity on SNU1 gastric cancer cells using the XTT assay, a sensitive method for assessing cell viability based on mitochondrial activity. Furthermore, to understand the molecular mechanisms underlying any observed cytotoxicity, we analyzed the levels of several key growth-related factors, including epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), mechanistic target of rapamycin (mTOR), extracellular signal-regulated kinase 1 (ERK1), and ERK2, using ELISA-based quantification. These signaling molecules play central roles in tumor cell proliferation, survival, and resistance to therapy [11]. Therefore, this study aimed to evaluate the cytotoxic potential of *Galium aparine* on the SNU1 gastric cancer cell line and to explore its impact on the expression of key proliferative signaling proteins. By characterizing these interactions, this study contributes to the growing body of research into plant-derived compounds as potential therapeutic agents for gastric cancer.

2. Experimental Methods

2.1 Cell Line and Culture Conditions

In this study, the SNU-1 human gastric carcinoma cell line (CRL-5971) was sourced from the American Type Culture Collection (ATCC, USA). The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin (Sigma-Aldrich), and incubated at 37 °C in a humidified atmosphere containing 5% CO₂.

Galium aparine L. plants at the flowering stage were collected from Yapıldac village (Ezine district, Çanakkale, Turkey; altitude 50–100 m). The taxonomic identification of the plant material was confirmed by Prof. H. Aşkın Akpulat (Department of Biology, Cumhuriyet University, Sivas, Turkey). A voucher specimen (CUFH-Voucher No: AA 7390) was deposited in the Herbarium of the Department of Biology, Cumhuriyet University, Sivas, Turkey. The collected plant material was air-dried under shade conditions and subsequently ground using a hammer mill to obtain a fine powder passing through a 200 µm sieve. One hundred grams of the powdered material were extracted with 500 mL of 65% ethanol at room temperature for 14 days in the dark with occasional shaking to ensure thorough solvent contact. Following extraction, the mixture was filtered using a vacuum filtration system and further clarified through a 0.42 µm filter paper. The solvent was removed under reduced pressure using a rotary evaporator to obtain a concentrated extract. The final extract concentration was adjusted to 60 mg/mL and stored at 4°C until use.

2.2 Viability Assay

The effect of *G. aparine* on SNU-1 cell viability was evaluated using the XTT assay. SNU-1 cells were seeded

into 96-well plates at a density of 1×10^4 cells per well and allowed to adhere overnight before treatment with *Galium aparine*. The cells were then exposed to varying concentrations of *G. aparine* (12.5, 25, 50, 100, and 200 µg/mL) for 24 hours, while cells treated with culture medium alone served as the control group. Following incubation, 50 µL of the XTT reagent was added to each well. After a 4-hour reaction time, the plates were gently shaken and absorbance was measured at 450 nm using a microplate reader (Thermo Fisher Scientific, Altrincham, UK). Cell viability was calculated as the percentage of viable cells relative to the untreated controls, with each experiment performed in triplicate. The IC₅₀ value was determined using GraphPad Prism 8.0 software by applying a non-linear regression model to the XTT assay data across the tested concentrations [12].

2.3 The measurement of mTOR, EGFR, ERK-1, ERK-2, and HER-2 levels

Commercial ELISA kits (BT Lab) were employed to measure the levels of selected growth factors. SNU-1 gastric cancer cells were exposed to 54.65 µg/mL of *Galium aparine* for 24 hours. Afterwards, both untreated control cells and *G. aparine*-treated cells were suspended in PBS at a final concentration of 1×10^6 cells/mL. The cell suspensions then underwent three freeze–thaw cycles, and the resulting supernatants were collected. The concentrations of mTOR, EGFR, ERK1, ERK2, and HER2 in these supernatants were quantified according to the manufacturer's protocol. Total protein levels in the cell lysates were quantified using a Bradford assay kit (SERVA, Germany). The growth factor concentrations obtained from the ELISA tests were then adjusted according to each sample's total protein content and reported as pg of target protein per mg of total protein [13].

2.4 Statistical Analysis

All experimental results are presented as the mean + standard error of the mean (SEM). For the cell viability assay, data were evaluated using one-way ANOVA followed by a suitable post hoc test. Comparisons of growth factor levels between groups were performed using an independent samples t-test. A p-value below 0.05 was considered statistically significant. All statistical analyses and graph preparations were carried out using GraphPad Prism version 8.0 (USA).

3. Results and Discussion

3.1 SNU-1 cell growth was inhibited by treatment with *Galium aparine*

The impact of *Galium aparine* on SNU-1 cell survival was assessed via the XTT assay. As presented in Figure 1, exposure to increasing concentrations of *G. aparine* (12.5–200 µg/mL) led to a clear, dose-dependent decline in cell

viability relative to the untreated controls. As presented in Figure 2, the IC₅₀ was calculated to be around 54.65 µg/mL. The highest tested dose (200 µg/mL) produced the greatest reduction in viability, whereas the lowest concentrations (12.5 and 25 µg/mL) did not cause any significant change. Overall, these results demonstrate that *G. aparine* reduces the viability of SNU-1 gastric cancer cells in a dose-dependent fashion ($p < 0.001$).

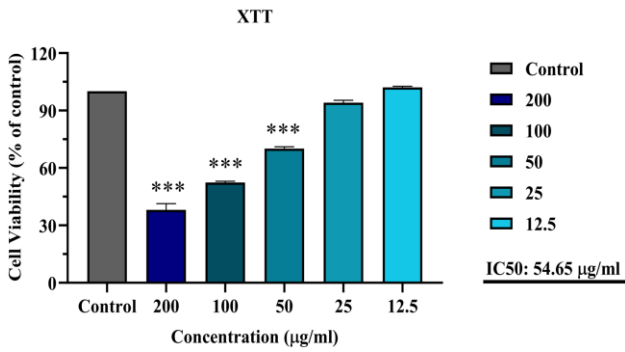


Figure 1. SNU-1 Cell Viability Following *G. aparine* Administration. This graph displays the mean + SEM of cell viability. Statistical significance (***) indicates a notable difference from the untreated control.

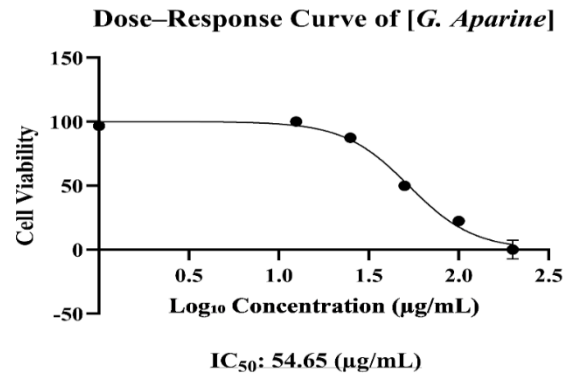


Figure 2. Log concentration–response curve of *G. aparine* in SNU-1 gastric cancer cells determined by XTT assay. Cell viability (%) was plotted against the logarithmic concentrations of *G. aparine* (12.5–200 µg/mL), and the IC₅₀ value was calculated using nonlinear regression analysis (GraphPad Prism 8.0).

3.2 Effects of *G. aparine* on Growth Factors in SNU-1 Cells

ELISA analysis revealed that a 24-hour exposure of SNU-1 cells to *G. aparine* (54.65 µg/mL) resulted in significant downregulation of growth factor-related proteins. Specifically, HER-2, EGFR, mTOR, ERK-1, and ERK-2 levels were significantly decreased compared to control cells ($p < 0.01$ – $p < 0.05$) (Figure 3).

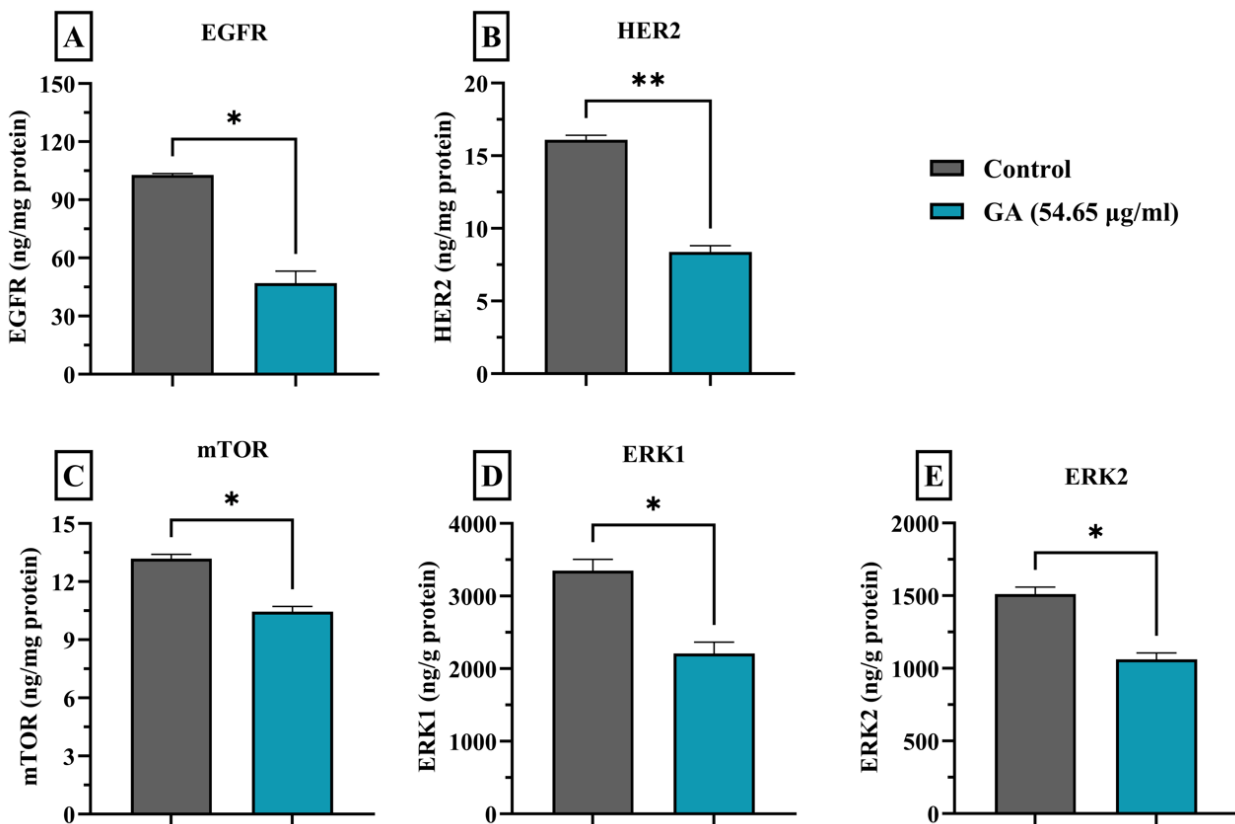


Figure 3. *G. aparine* Reduces Growth Factor-Related Protein Levels in SNU-1 Cells. This figure illustrates the mean + SEM quantities of HER-2, EGFR, mTOR, ERK-1, and ERK-2 proteins, determined via ELISA, following exposure to *G. aparine* (54.65 µg/mL). Statistical significance relative to the control group is indicated by * $p < 0.05$ and ** $p < 0.01$.

To our knowledge, this study is the first to investigate the anti-cancer effect of Galium aparine (GA) on the human gastric carcinoma SNU1 cell line. Although Galium aparine has a long history of ethnopharmacological use and has been investigated for various biological activities, its direct cytotoxic effects on gastric cancer cells, specifically SNU1, have not been reported previously. This highlights the novelty of our findings and fills an important gap in the exploration of Galium species as potential sources of anti-cancer compounds. Korkmaz et al. reported that ethanol extracts of *G. aparine* produced marked, dose-dependent cytotoxicity in the A549 lung cancer cell line [9]. Moreover, Galium verum at a concentration of 400 µg/mL was shown to reduce cell viability, promote apoptosis, elevate the BAX/BCL2 ratio and ROS levels, and cause cell cycle arrest at the G0/G1 phase in HT29 colon cancer cells. Additionally, Atmaca et al. demonstrated that the methanolic extract of *G. aparine* may exert anti-cancer effects on breast cancer cells while sparing normal breast epithelial cells [14, 15]. "The abundant flavonoids and polyphenols present in Galium species are closely linked to their notable anticancer, immunomodulatory, and antimicrobial properties. Various Galium species also contain iridoid compounds, which contribute to their anti-inflammatory and anticancer actions. In addition, anthraquinones identified in Galium plants are known for their antimicrobial and tumor-inhibiting effects. Phytosterols found in these species can help lower blood cholesterol levels and have demonstrated antitumor potential [16]. Zhao et al. found that diosmetin, isolated from *G. verum*, showed significant anti-cancer activity. In their study, mice treated with this compound experienced a dose-dependent reduction in tumor growth, with higher doses leading to greater tumor inhibition [17].

In this study, the XTT assay demonstrated that *G. aparine* has a clear, dose-dependent cytotoxic effect on SNU1 cells, with an IC50 of 54.65 µg/mL. This result is consistent with the earlier in vitro findings described above, which demonstrated similar cytotoxic effects in other tumor cell lines. These findings support the hypothesis that *G. aparine* may exert its effects through pathways that compromise cell viability, potentially mediated by its rich phytochemical profile, which includes iridoids, flavonoids, and phenolic compounds. We investigated how *G. aparine* affects the biological behavior of SNU-1 cells by examining the levels of proteins crucial for cell growth and programmed cell death. A key focus was the Epidermal Growth Factor Receptor (EGFR), a receptor tyrosine kinase vital for numerous cellular functions and a prime target in cancer therapy. Overexpression and activation of EGFR are frequently observed in various cancers, including gastric cancer, where they drive the proliferation, migration, and invasion of cancerous cells. When phosphorylated, EGFR can trigger several important signaling cascades, such as the ERK1/ERK2, PI3K/AKT/mTOR, and MAPK/MEK/ERK pathways [13].

The HER2 receptor is a critical therapeutic target across several cancer types, notably breast, colorectal, and gastric cancers. Approximately 20% of invasive breast cancers, 22% of gastric cancers, and 6% of colorectal cancers exhibit either HER2 protein overexpression or HER2 gene amplification. HER2 primarily relays its signals through key survival pathways: the phosphatidylinositol 3-kinase (PI3K)/Akt pathway and the Erk1-2/MAPK pathway [18]. The PI3K/AKT/mTOR signaling pathway is often abnormal in various diseases, particularly in cancer, and specifically in gastric cancer (GC). This pathway is crucial for several key cancer-related processes, including cell growth, survival, proliferation, and programmed cell death (apoptosis). Its improper function in GC not only prevents cell death but also encourages undesirable characteristics like resistance to chemotherapy, the spread of cancer (metastasis), new blood vessel formation (angiogenesis), and epithelial-mesenchymal transition (EMT). The dysregulation of this pathway is directly linked to both the development and prognosis of gastric cancer [19, 20]. Research indicates that ERK-1, ERK-2, and ERK-3 protein levels are elevated in gastric cancer (GC) tissues when compared to healthy adjacent mucosal tissue. This suggests that the upregulation of the ERK pathway contributes to the proliferation and transformation of gastric mucosal cells. Therefore, it's believed that the ongoing activation of the MAPK/ERK pathways plays a significant role in the development of gastric cancer [21]. In the current study, our ELISA-based analyses showed that *G. aparine* treatment significantly reduced the levels of EGFR, HER2, mTOR, ERK1, and ERK2 in SNU1 cells compared to untreated controls, as illustrated in the growth factor figure. This suppression of critical growth and survival pathways strongly supports the observed cytotoxicity and suggests that *G. aparine* may induce apoptosis or cell cycle arrest by downregulating oncogenic signaling pathways. To our knowledge, this is the first evidence indicating that *G. aparine* can modulate these specific targets in gastric cancer cells. In line with our results, Atmaca's research showed that Galium aparine markedly lowers phospho-ERK1/2 protein levels in MDA-MB-231 and MCF-7 breast cancer cells, indicating that *G. aparine* may possess compounds with promising anti-tumor and anti-angiogenic effects in breast cancer [22]. Taken together, our findings indicate that *G. aparine* demonstrates a promising anti-cancer effect against SNU1 gastric cancer cells by inhibiting cell proliferation, as shown by the XTT assay, an effect that may be mediated through the downregulation of key growth factors involved in oncogenesis. This potential mechanism supports the therapeutic relevance of *G. aparine*, especially in the context of combination strategies targeting multiple cancer pathways. Although the present study provides novel insights into the antiproliferative effects of Galium aparine in SNU-1 gastric cancer cells, several limitations should be acknowledged. First, the experiments were conducted using a single gastric cancer cell line. Gastric cancer is a highly heterogeneous disease with diverse molecular and histopathological subtypes;

therefore, the use of only the SNU-1 cell line may limit the generalizability of our findings. In addition, the absence of a normal gastric epithelial cell line prevents evaluation of the selectivity of *G. aparine* toward cancerous versus non-cancerous cells. Future studies should include additional gastric cancer cell lines as well as normal gastric epithelial cell models to validate these findings and to better assess treatment selectivity and safety. Furthermore, in vivo models and mechanistic investigations at the gene expression level would provide a more comprehensive understanding of the therapeutic potential of *G. aparine*.

In conclusion, this study is the first to demonstrate that *Galium aparine* exerts significant anti-cancer effects on SNU1 gastric cancer cells through a combination of direct cytotoxicity and suppression of major growth signaling pathways, including EGFR, HER2, mTOR, ERK1, and ERK2. These findings contribute valuable evidence supporting the potential of *G. aparine* as a candidate for further development in gastric cancer therapy.

Conflict of Interest

There are no conflicts of interest in this work.

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Declaration of Generative AI

The authors did not use any generative AI or AI-assisted technologies in the preparation of this manuscript, including the data analysis and writing stages

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