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Cytotoxic Evaluation of *Acanthophyllum glandulosum* Bung. ex Boiss: Comparative Analysis of Anticancer Activity on AGS Gastric Cancer Cells and Human dermal fibroblasts

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ABSTRACT

Introduction: Gastric cancer remains one of the most prevalent and lethal cancers worldwide. In the quest for novel therapeutic agents, medicinal plants have garnered significant interest. This study evaluates the cytotoxic effects of Acanthophyllum glandulosum Bung. ex Boiss, a plant from the Caryophyllaceae family, on AGS (gastric cancer) and HDF (human fibroblast) cell lines.

Methods: *Acanthophyllum glandulosum* samples were collected from Chaharmahal and Bakhtiari Province, identified, and authenticated. Hydroalcoholic extracts were prepared using a maceration technique. The cytotoxicity of these extracts was assessed using the MTT assay on AGS and HDF cell lines. IC50 values were calculated, and statistical analysis was performed to evaluate the data.

Results: The IC50 value for AGS cells was 294 µg/mL, with a 95% Confidence Interval (CI) of 184-470 µg/mL and an R-value of 0.8309. For HDF cells, the IC50 was 74 µg/mL, with a 95% CI of 70-78 µg/mL and an R-value of 0.9964.

Conclusion: *Acanthophyllum glandulosum* demonstrated significant cytotoxicity against AGS cells, inhibiting cell growth at concentrations below 100 µg/mL. However, it also exhibited cytotoxic effects on HDF cells, suggesting that while it has potential as an anticancer agent, further studies are necessary to evaluate its selectivity and minimize potential toxicity to non-cancerous cells.

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Introduction

Gastric cancer continues to pose a significant global health challenge, ranking among the most prevalent and deadly forms of cancer worldwide. According to recent epidemiological reports, gastric cancer remains a leading cause of cancer-related mortality, accounting for a substantial number of deaths each year, particularly in East Asia, Eastern Europe, and parts of Central and South America (Sung et al., 2021). Despite advances in diagnostic techniques and therapeutic strategies, the prognosis for gastric cancer patients often remains poor,

primarily due to late-stage diagnosis and the high likelihood of metastasis. Additionally, the current treatment modalities, which include surgery, chemotherapy, and radiation therapy, are often associated with severe side effects and limited effectiveness, particularly in advanced stages of the disease. These challenges underscore the critical need for novel therapeutic approaches that can improve patient outcomes while minimizing adverse effects.

In recent years, the exploration of natural products, particularly medicinal plants, has gained considerable attention as a potential source of new and effective cancer therapies. Medicinal plants are rich in diverse bioactive compounds that have demonstrated various therapeutic properties, including anti-cancer, anti-inflammatory, and antioxidant activities. One such plant of interest is *Acanthophyllum glandulosum* Bung. ex Boiss, commonly known as Chubak Nekaee, which belongs to the Caryophyllaceae family. This plant has a long history of use in traditional medicine, particularly in the treatment of gastrointestinal disorders. Its therapeutic potential is largely attributed to the presence of saponins and gum-like substances, which have been shown to possess significant emulsifying properties (Chandra and Rawat, 2015; Gaidi et al., 2000; Jahanbin, 2018).

The pharmacological properties of *Acanthophyllum glandulosum* are not limited to its traditional use. Several studies have reported its antioxidant activity, which is a critical factor in preventing cellular damage caused by oxidative stress, a known contributor to cancer development and progression. The antioxidant properties of this plant are believed to be linked to its high content of polyphenolic compounds, which can neutralize free radicals and thereby reduce oxidative stress (Gaidi et al., 2000; Jahanbin, 2018; Naghibi et al., 2014). In addition to its antioxidant effects, certain species within the Acanthophyllum genus have demonstrated anti-inflammatory and immunomodulatory activities, which further contribute to their potential as therapeutic agents. These properties are particularly relevant in the context of cancer treatment, as inflammation and immune system dysregulation play pivotal roles in cancer progression.

Moreover, recent studies have highlighted the cytotoxic effects of Acanthophyllum species on various cancer cell lines, suggesting their potential as anti-cancer agents. For instance, research has shown that extracts from different Acanthophyllum species can induce cell death in other cancer cell lines, while sparing normal cells, indicating selective cytotoxicity (Mosaddegh et al., 2018). This selective action is a crucial characteristic for any potential cancer treatment, as it minimizes damage to healthy tissues and reduces the likelihood of adverse side effects.

Given the promising therapeutic properties of *Acanthophyllum glandulosum*, the present study aims to explore its cytotoxic effects specifically on AGS (gastric cancer) and HDF (human fibroblast) cell lines. The AGS cell line, derived from human gastric adenocarcinoma, is widely used as a model for studying gastric cancer biology and testing anti-cancer agents. On the other hand, HDF cells serve as a model for normal human fibroblasts, providing a benchmark for assessing the selective toxicity of potential therapeutic agents. The primary objective of this study is to evaluate the differential cytotoxicity of *Acanthophyllum glandulosum* extracts on cancerous versus normal cells, which could provide valuable insights into its potential as a therapeutic agent in the treatment of gastric cancer.

By investigating the cytotoxic effects of *Acanthophyllum glandulosum* on these cell lines, this study seeks to contribute to the growing body of research on natural products as alternative treatments for cancer. The findings could pave the way for further research and development of plant-based therapies that offer a safer and more effective option for patients with gastric cancer.

Materials and Methods

Collection, Identification, and Extraction of Plants

In this study, plant specimens were collected from various regions within Chaharmahal and Bakhtiari province, known for its rich biodiversity. The identification and verification of the collected plants were conducted by Dr. Shirmardi at the Agricultural Research Center of the province. Each plant specimen was preserved and cataloged in the herbarium of the Medicinal Plants Research Center at Shahrekord University of Medical Sciences, where they were assigned specific herbarium codes for future reference.

The plant materials were carefully dried in the shade to prevent degradation from direct sunlight, ensuring the preservation of active compounds. Once adequately dried, the samples were ground into a fine powder using an electric mill. Hydroalcoholic extracts were prepared using the maceration method. This process was repeated 1-3 times to ensure maximum extraction of bioactive compounds. A mixture of water and ethanol (without the addition of butyric acid) was used as the solvent, in specific ratios tailored to optimize extraction efficiency. The resulting extract was filtered through filter paper, and the filtrate was incubated at 37°C for 3 to 5 days. This incubation period allowed for the evaporation of the solvent, leaving behind a concentrated extract in either a pasty or powdered form. The concentrated extracts were then dissolved in dimethyl sulfoxide (DMSO), centrifuged to remove any precipitates, and subsequently prepared for use in experimental assays.

Cell Culture and Maintenance

AGS (gastric adenocarcinoma) and HDF (human dermal fibroblast) cell lines were selected for this study to

evaluate the cytotoxic effects of the plant extracts. AGS cells, representing a model for gastric cancer, were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 1% L-glutamine. The HDF cells, serving as a model for normal human fibroblasts, were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% FBS, 1% penicillin-streptomycin, and 1% L-glutamine. Both cell lines were maintained in a humidified atmosphere of 5% CO2 at 37°C.

For passaging, cells were detached using 0.25% trypsin-EDTA solution once they reached approximately 80-90% confluence. Cells were then reseeded at appropriate densities for further experiments. Before each experiment, cell viability was confirmed using trypan blue exclusion assay to ensure that only healthy cells were utilized.

Assessment of Anticancer Effects Using the MTT Assay

To evaluate the anticancer potential of the plant extracts, the MTT assay was employed. This colorimetric assay measures cellular metabolic activity as an indicator of cell viability, proliferation, and cytotoxicity. The AGS and HDF cells were seeded into 96-well plates at densities ranging from 1,000 to 10,000 cells per well, depending on cell type and size. After an incubation period of 24 hours in a CO2 incubator (5% CO2, 95% humidity), allowing the cells to adhere and achieve exponential growth, the cells were treated with varying concentrations of the plant extracts (ranging from 10 to 1,000 µg/mL). The extracts were first dissolved in DMSO and then diluted with RPMI-1640 or DMEM medium, depending on the cell type, ensuring that the final concentration of DMSO in the culture medium did not exceed 0.1%, to avoid solvent-induced cytotoxicity.

Following treatment, cells were incubated for 24 hours, depending on the experimental design. Post-incubation, 20 µL of MTT solution (5 mg/ml in PBS) was added to each well and incubated for 3-4 hours at 37°C. The MTT solution was then removed, and the resulting formazan crystals were dissolved in 150 µL of DMSO. Absorbance was measured at 570 nm using a microplate reader, with a reference wavelength of 630 nm. The percentage of viable cells was calculated relative to untreated controls, allowing for the determination of the IC50 values for each extract.

Statistical Analysis

To assess the cytotoxic effects of *Acanthophyllum glandulosum* on AGS and HDF cell lines, statistical analyses were performed using SPSS software (version 17). The comparison between treated groups and the untreated control group for each cell line was conducted using an independent samples t-test, with significance set at $p < 0.05$. The Bonferroni correction was applied to adjust for multiple comparisons. For overall comparisons among different treatment concentrations, one-way ANOVA followed by Tukey's post hoc test was employed. Additionally, to compare the effects between AGS and HDF cell lines, two-way ANOVA was utilized to analyze the interaction between cell line type and treatment. Results were considered statistically significant at $p < 0.05$.

Results

The cytotoxic effects of *Acanthophyllum glandulosum* on AGS (gastric adenocarcinoma) and HDF (human dermal fibroblast) cell lines were assessed by calculating the IC50 values, which represent the concentration of the extract required to inhibit cell viability by 50%. The statistical parameters for each cell line are detailed below:

AGS Cell Line:

IC50 Value: The IC50 for the AGS cell line was determined to be 294 µg/mL. This value represents the concentration at which the extract inhibits 50% of cell viability in AGS cells.

95% Confidence Interval (CI): The IC50 estimate is accompanied by a 95% CI ranging from 184 µg/mL to 470 µg/mL. This range indicates the uncertainty around the IC50 estimate and suggests variability in the response of AGS cells to the extract.

Coefficient of Determination (R^2): The R^2 value for the dose-response curve was 0.8309, indicating a strong fit of the data to the model used to estimate the IC50. An R^2 value closer to 1 signifies a high degree of correlation between the observed and predicted values.

HDF Cell Line:

IC50 Value: For the HDF cell line, the IC50 was found to be 74 µg/mL. This lower IC50 compared to AGS cells indicates a higher sensitivity of HDF cells to the extract.

95% Confidence Interval (CI): The IC50 estimate is supported by a narrow 95% CI ranging from 70 µg/mL to 78 µg/mL. The tight CI reflects a high precision in the IC50 estimate and minimal variability in the response of HDF cells to the extract.

Coefficient of Determination (R^2): The R^2 value was 0.9964, demonstrating an excellent fit of the dose-response data to the model. This high R^2 value suggests a very strong correlation between the observed data and the model predictions.

Statistical Comparison:

To compare the cytotoxic effects of the extract on AGS versus HDF cell lines, an independent samples t-test was

performed on the IC50 values. This test was used to determine whether the observed differences in IC50 values between the two cell lines are statistically significant.

The comparison revealed a statistically significant difference in IC50 values between AGS and HDF cell lines ($p < 0.05$). Specifically, the significantly lower IC50 value for HDF cells indicates that the plant extract has a higher cytotoxic effect on normal fibroblast cells compared to the cancerous AGS cells.

Discussion

The primary objective of this study was to investigate the cytotoxic effects of *Acanthophyllum glandulosum* on AGS (gastric adenocarcinoma) and HDF (human dermal fibroblast) cell lines and to evaluate its potential as an anti-cancer agent. The findings indicate that while the extract has significant cytotoxic effects, its lack of selectivity raises concerns about its therapeutic potential.

The IC50 value for *Acanthophyllum glandulosum* against AGS cells was found to be 294 µg/mL, with a 95% confidence interval ranging from 184 to 470 µg/mL. This moderate cytotoxicity is similar to results reported by Mosaddegh et al. (2018), who observed comparable IC50 values for various plant extracts against different cancer cell lines, suggesting a moderate efficacy (Mosaddegh et al., 2018). These findings suggest that *Acanthophyllum glandulosum* may have promising, though not exceptional, anti-cancer properties.

In contrast, the IC50 value for HDF cells was significantly lower at 74 µg/mL, with a 95% confidence interval of 70 to 78 µg/mL. This indicates a higher cytotoxic effect on normal fibroblasts. This result aligns with Asadi-Samani et al. (2019), who reported that certain medicinal plants exhibit broad-spectrum cytotoxicity, affecting both cancerous and non-cancerous cells (Asadi-Samani et al., 2019). Calderón-Montaño et al. (2021) also found that plant extracts with broad-spectrum activity pose challenges for achieving selectivity (Calderón-Montaño et al., 2021). Such broad-spectrum activity could limit the therapeutic window of *Acanthophyllum glandulosum*, making it less suitable for selective cancer therapy. The lack of selectivity observed in our study is consistent with Jiménez-González et al. (2023), who found that many plant extracts with significant cytotoxicity often lack the required selectivity for cancer cells over normal cells (Jiménez-González et al., 2023). Garcia-Oliveira et al. (2021) emphasized the importance of developing selective compounds to minimize adverse effects on normal cells (Garcia-Oliveira et al., 2021).

The strengths of this study include the comprehensive assessment of *Acanthophyllum glandulosum* on both cancerous and normal cell lines, providing a broad view of its cytotoxic profile. The use of multiple concentrations and rigorous statistical analysis adds robustness to the findings. However, the study has limitations. The high IC50 values for AGS cells suggest that the extract's potency may be moderate, requiring further refinement. Additionally, the significant cytotoxicity observed in HDF cells indicates a lack of selectivity, which could impact the extract's therapeutic potential.

Future research should focus on improving the selectivity of *Acanthophyllum glandulosum* extracts to minimize toxicity to normal cells while enhancing efficacy against cancer cells. Investigating the specific bioactive compounds and their mechanisms of action could provide valuable insights into optimizing the extract’s therapeutic potential. In vivo studies are crucial to assess the pharmacokinetics, toxicity, and overall efficacy of the extract. Additionally, exploring combination therapies or formulation strategies that could enhance the therapeutic index of the extract while reducing off-target effects should be considered.

Conclusion

Based on the study results, the plant extract demonstrates a potent cytotoxic effect against the AGS gastric cancer cell line, with an IC50 value of 294 µg/ml and a 95% confidence interval ranging from 184 to 470 µg/ml, showing considerable potential for cancer treatment. This is in contrast to its IC50 value of 74 µg/ml (with a 95% confidence interval from 70 to 78 µg/ml) observed against the HDF human fibroblast cell line, indicating significant cytotoxicity even in non-cancerous cells. The broader cytotoxic effects observed in HDF cells suggest that while the plant extract may offer therapeutic benefits against gastric cancer, its non-selective activity could limit its potential for clinical application in cancer therapy. Further investigations are necessary to refine the extract's selective activity and assess its safety and efficacy in more detail, to identify if modifications or combination therapies could enhance its specificity and therapeutic index.

Declarations

Conflict of interest

The authors declare no conflict of interest.

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Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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