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Potential Effects of *Alcea glabrata* Methanolic Extract on Gastric Adenocarcinoma (AGS) Cells: Insights into *HER2* and Apoptosis-related Gene Expression

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ABSTRACT

Gastric cancer is a significant global health issue characterized by a high mortality rate. Recent studies highlight the potential of plant-derived compounds in targeting molecular pathways involved in cancer. This study aimed to evaluate the apoptotic and anticancer effects of *Alcea glabrata* methanolic extract on gastric cancer cells, focusing on the modulation of *HER2, HSP90a, P53*, and *Caspase-3* gene expression.

The methanolic extract was prepared from the aerial parts of *Alcea glabrata*. Its antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Human gastric adenocarcinoma (AGS) and human gum fibroblast (HUGU) cell lines were treated with different concentrations of the extract. Cell viability was determined using the MTT assay, and IC_{50} values were calculated. Apoptosis was analyzed by flow cytometry, and real-time PCR was used to quantify the expression levels of *HER2, HSP90a, P53*, and *Caspase-3*.

The extract exhibited potent antioxidant activity, as demonstrated by the DPPH assay. It reduced cell viability in both AGS and HUGU cell lines, with a lower IC_{50} in AGS cells, indicating selective toxicity toward cancer cells. Flow cytometry revealed a significant increase in apoptosis in AGS cells. Additionally, gene expression analysis showed upregulation of *P53* and C*aspase-3,* and downregulation of *HER2* in AGS cells. Changes were less marked in HUGU cells. The extract also demonstrated vigorous antioxidant activity.

Alcea glabrata extract exhibited selective anticancer effects on AGS cells by inducing apoptosis and modulating gene expression. Its antioxidant properties and downregulation of *HER2* suggest its potential as a complementary treatment agent for gastric cancer.

Keywords: *Alcea glabrata*; Apoptosis; Gastric adenocarcinoma; *HER2;* Methanolic extract

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INTRODUCTION

Gastric cancer (GC) remains one of the most significant global health burdens, ranking among the top causes of cancer-related morbidity and mortality. According to global cancer statistics, GC is the third leading cause of cancer-associated deaths, with approximately 1 million new cases diagnosed annually.[1] Despite advances in diagnostic tools and therapeutic approaches, the overall prognosis for GC remains poor, mainly due to late-stage diagnosis and the aggressive nature of the disease. Early-stage gastric cancer often presents with nonspecific and vague symptoms, which contributes to delays in diagnosis. The conventional treatment modalities for GC, including surgery, chemotherapy, and radiotherapy, are commonly employed but are often associated with limited efficacy, significant side effects, and the development of resistance over time.[2–4]

These limitations underscore the urgent need to develop alternative or complementary therapeutic strategies with improved safety and efficacy. In recent years, there has been growing interest in the use of medicinal plants as sources of anticancer agents. Medicinal plants are rich in diverse bioactive compounds, such as flavonoids, alkaloids, terpenoids, and phenolic compounds, which exhibit anticancer properties through various mechanisms. These include the induction of apoptosis, inhibition of cancer cell proliferation, suppression of angiogenesis and metastasis, and modulation of key signaling pathways involved in tumor development.[5,6]

Moreover, many plant-derived compounds possess antioxidant and anti-inflammatory activities that can reduce oxidative stress and chronic inflammation, major contributors to carcinogenesis.[7,8]

One such plant with promising pharmacological potential is *Alcea glabrata* (*A. glabrata*), a species native to the *Malvaceae* family, which is found in parts of Asia and the Mediterranean region, particularly Iran.[9] Traditionally, *A. glabrata* has been used for its anti-inflammatory, antimicrobial, and wound-healing properties. Preliminary studies on related species suggest that extracts of *Alcea* may exert anticancer effects by inducing apoptosis and suppression of tumor cell viability. However, the molecular mechanisms underlying these effects, particularly in gastric cancer, remain largely unexplored.[10–12] In gastric cancer, dysregulation of key molecular pathways is central to disease progression. The overexpression of human epidermal growth factor receptor 2 (HER2), a receptor tyrosine kinase, has been observed in approximately 20% of GC cases and is associated with aggressive tumor behavior and poor clinical outcomes.[13,14] Likewise, heat shock protein 90 alpha (HSP90α), a molecular chaperone, is upregulated in many solid tumors and contributes to the stabilization of multiple oncogenic proteins, thus promoting tumor survival under stress conditions.[15] In contrast, tumor protein p53 (*TP53*), one of the most frequently mutated suppressor genes in cancer, plays a pivotal role in inducing cell cycle arrest and apoptosis in response to DNA damage.[16,17] Activation of downstream effectors such as Caspase-3, a key executioner of the apoptotic cascade, is a hallmark of programmed cell death.[18]

Given the promising pharmacological profile of *A. glabrata* and the critical roles of *HER2*, *HSP90α*, *P53*, and *Caspase-3* in gastric tumor biology, the present study aimed to evaluate the cytotoxic, antioxidant, and pro-apoptotic effects of *A. glabrata* methanolic extract on human gastric adenocarcinoma (AGS) cells. Furthermore, we examined the extract's ability to modulate the expression of these molecular targets to elucidate potential mechanisms of action. By comparing responses in both cancerous and normal cells, this study seeks to determine the therapeutic selectivity of *A. glabrata*, supporting its potential role as a complementary treatment for gastric cancer.

MATERIALS AND METHODS

Preparation of Plant Extract

Ten grams of dried aerial parts of *A. glabrata* were obtained from the Plant Bank of the Iranian Biological Resource Center (IBRC), Karaj, Iran. For extract preparation, 70% methanol (Merck, Germany) was added to the powdered plant material, and the mixture was incubated at 40°C for 24 hours. The extract was stored at 2–8°C until it was used in the following experiments.

Assessment of Antioxidant Activity Using the DPPH Method

The antioxidant activity of *A. glabrata* extract was assessed using the DPPH assay. For this purpose, mixtures containing various concentrations of the

extract and 1 mmol/L of the methanolic DPPH solution were prepared. After 20 minutes of incubation in the dark, absorbance was measured at 517 nm using a spectrophotometer (PG Instruments, China), with ascorbic acid used as the antioxidant control. The percentage of DPPH inhibition was determined using the following formula:

DPPH Inhibition (%) = [(Control Absorbance − Sample Absorbance) ÷ Control Absorbance] × 100

This calculation reflects the antioxidant activity of the extract based on the reduction in absorbance and the corresponding color change of the solution.

Preparation and Culture of Cell Lines

Two cell lines were used in this study: the human gum fibroblast cell line (HUGU; IBRC C10459) and the human gastric adenocarcinoma cell line (AGS; IBRC C10071), both obtained from the Human and Animal Cell Bank at the IBRC in Tehran, Iran. Cells were cultured in DMEM:F12 medium (Gibco, UK) supplemented with 10% fetal bovine serum (FBS) (Gibco, UK) and 2 mmol/L L-glutamine (Sigma, USA) and maintained at 37°C with 5% CO_2.

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) Assay for Cell Viability

To assess the cytotoxic effect of *A. glabrata* on cell viability, HUGU and AGS cells were seeded into 96-well plates at densities of 1×10^4 and 8×10^3 cells/well, respectively. After 24 hours of incubation, cells were treated with serial dilutions of the extract (ranging from 2.5 to 1280 µg/mL) and then incubated for an additional 48 hours. MTT solution (Sigma, USA) was then added to each well, followed by 3 hours of incubation. The formazan crystals were dissolved in dimethyl sulfoxide (DMSO) (Sigma, USA), and absorbance was determined at 570 nm using a Biotek ELX800 microplate reader (USA). The MTT assay was conducted in triplicate.

Flow Cytometric Analysis of Apoptosis

AGS and HUGU cells were seeded into 6-well plates and incubated overnight at 37°C in a 5% CO_2 incubator (New Brunswick, USA). The cells were treated with 500 µg/mL of *A. glabrata* extract for 48 hours, while control wells remained untreated. After washing with phosphate-buffered saline (PBS) (Gibco, UK), cells were detached using Trypsin-EDTA (Gibco, UK), stained with Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI), and analyzed by flow cytometry using a BD FACS-Calibur system (Becton Dickinson, USA). Data processing was performed with FlowJo software (TreeStar, Ashland, USA) to determine the percentage of apoptotic and necrotic cells.

Gene Expression Analysis by Real-Time PCR

To evaluate the effect of *A. glabrata* extract on gene expression, AGS and HUGU cells were treated with 500 µg/mL of the extract for 48 hours. Total RNA was extracted using the RNX Plus kit (CavoshClone, Iran), and cDNA was synthesized using the RT ROSET kit (South Korea). Real-time PCR was then performed with SYBR Green Master Mix (Biofact, South Korea) on an ABI StepOne system (Applied Biosystems, USA). The expression levels of *HER2*, *HSP90α*, *P53*, and *Caspase-3* genes were assessed. *GAPDH* was selected as the internal reference gene because its expression remained stable under all experimental conditions. Relative levels of gene expression were determined using the $2^{-\Delta\Delta Ct}$ method, where Ct values of target genes were normalized to *GAPDH* ($\Delta Ct = Ct_{target} - Ct_{GAPDH}$), and then compared with those of the untreated control group ($\Delta\Delta Ct = \Delta Ct_{treated} - \Delta Ct_{control}$). All reactions were carried out in triplicate, and the results are presented as mean±SD. A list of primer sequences used in this study is presented in Supplementary Table 1.

Statistical Analysis

Statistical analyses were performed using SPSS software (version 25). Data are presented as mean±SD obtained from at least three independent experiments. For the DPPH assay, statistical differences among multiple concentrations were assessed using one-way analysis of variance (ANOVA), followed by Tukey post hoc test. In the MTT assay, the combined effects of extract concentration and cell type were evaluated using two-way ANOVA, with Sidak's multiple comparisons test applied for post hoc analysis. Comparisons between treated and control groups in the apoptosis and gene expression assays were conducted using an unpaired Student *t* test. Relative gene expression levels were initially calculated using Genex 6 software, and the resulting data were subsequently subjected to statistical analysis in SPSS. All graphical representations were generated using GraphPad Prism 8. A $p<0.05$ was considered indicative of statistical significance.

RESULTS

Antioxidant Potential of *A. glabrata* Evaluated by DPPH Assay

The antioxidant potential of the methanolic extract of *A. glabrata* was assessed at various concentrations (100, 250, and 500 µg/mL) and compared with ascorbic acid, which served as the positive control. As illustrated in Figure 1, the extract demonstrated a concentration-dependent antioxidant effect, with higher concentrations resulting in increased DPPH radical scavenging activity. Notably, DPPH method results showed that about 293 µg/mL of *A. glabrata* extract could remove 50% of free radicals.

Cytotoxic Effects of *A. glabrata* on the Cell Lines Assessed by MTT Assay

The cytotoxic activity of the methanolic extract of *A. glabrata* was evaluated using the MTT assay on AGS and HUGU cells. The results demonstrated a concentration-dependent inhibition of cell proliferation, with a significantly more substantial inhibitory effect observed in the AGS cell line compared to the HUGU cells (Figure 2). After 48 hours of treatment, the IC_{50} value for AGS cells was approximately 500 µg/mL, while a significantly higher IC_{50} value of 1280 µg/mL was observed for the HUGU cell line. These findings indicate that *A. glabrata* extract exhibits selective cytotoxicity toward gastric adenocarcinoma cells, with minimal toxicity to normal cells.

Induction of Apoptosis by *A. glabrata* Extract in the Cells: Flow Cytometry Analysis

After 48 hours of treatment with 500 µg/mL of *A. glabrata* methanolic extract, morphological changes indicative of apoptosis were observed in AGS and HUGU cells under an inverted microscope (Figure 3).

Based on these observations, the apoptotic effects of the extract were further evaluated in the cells using flow cytometry. Apoptosis was quantified by Annexin V-FITC/PI staining. AGS cells showed 43.93% apoptosis and 0.129% necrosis following treatment. In contrast, untreated control AGS cells showed 12.36% apoptosis and 0.351% necrosis (Figure 4A–B). These results show a significant induction of apoptosis in AGS cells upon treatment. In the HUGU cell line, exposure to the same extract concentration resulted in 11.07% apoptosis and 0.103% necrosis, whereas the untreated control cells exhibited 3.09% apoptosis and 0.362% necrosis (Figure 4C–D).

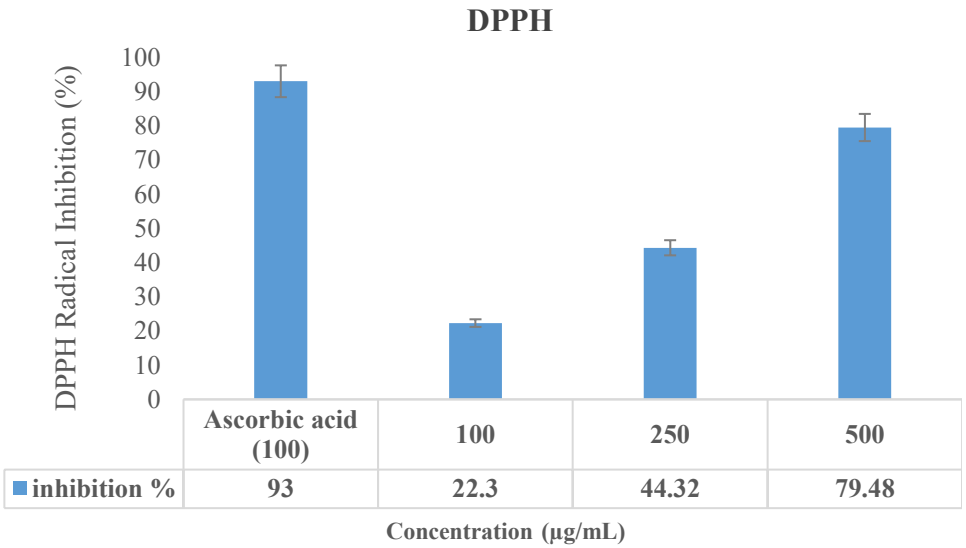

Figure 1. DPPH-based assessment of antioxidant activity in *A. glabrata* extract. DPPH test results in evaluating the antioxidant activity of *A. glabrata* extract showed a concentration-dependent antioxidant effect. In this method, ascorbic acid was used as a standard. Data represent mean±SD of three independent experiments.

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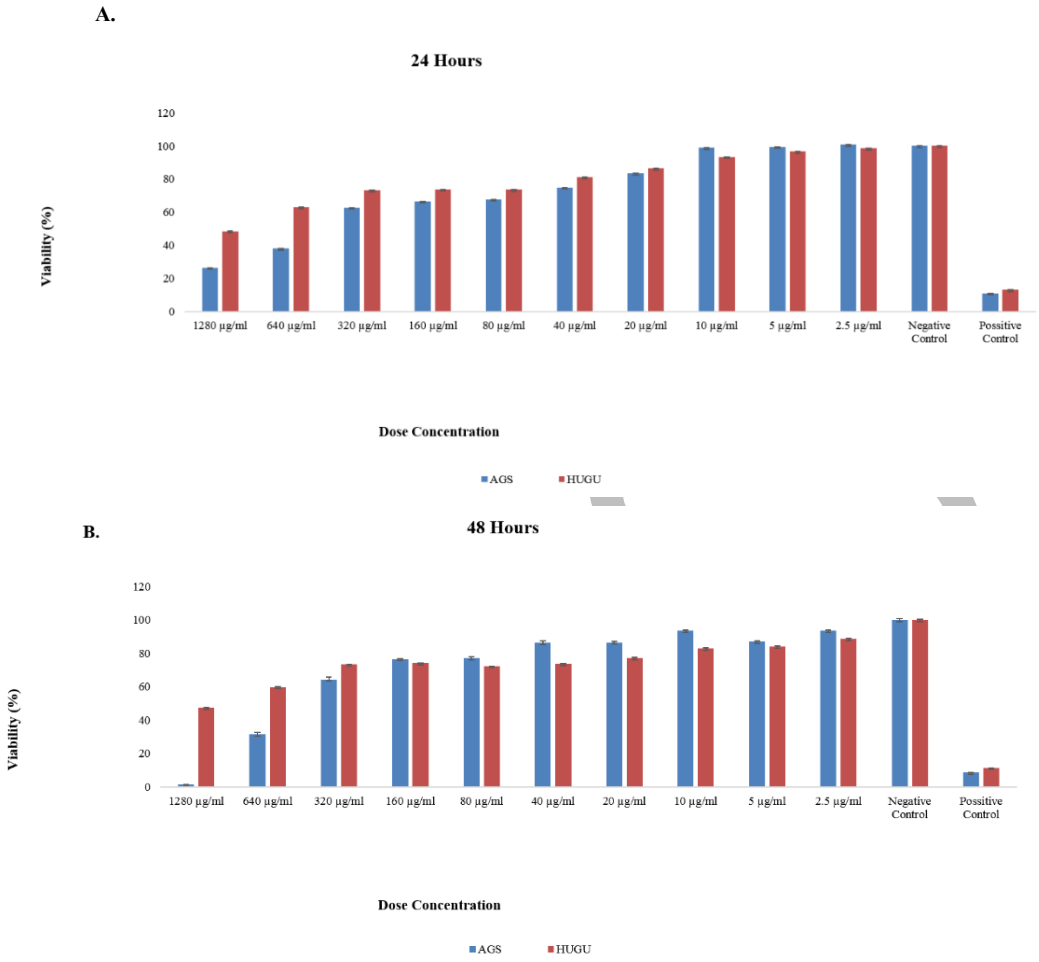

Figure 2. Comparative analysis of cell viability in response to *A. glabrata*. Cell survival rates of AGS and HUGU cells were assessed after 24 hours (A) and 48 hours (B) of treatment with varying concentrations of *A. glabrata* extract. The extract induced a greater decrease in viability in AGS cells compared to normal HUGU cells in a time- and dose-dependent manner. Data are expressed as the mean±SD of three independent experiments conducted in triplicate.

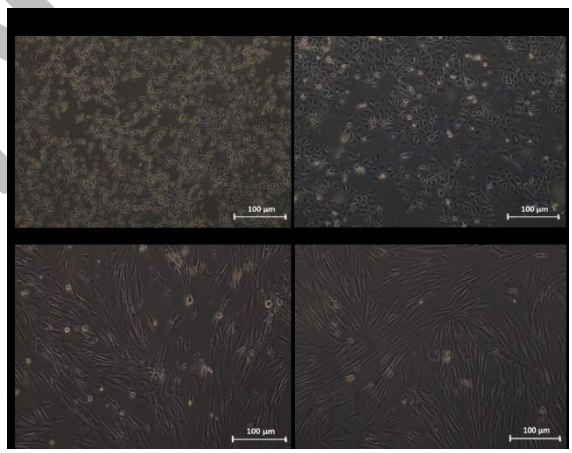

Figure 3. Morphological changes in cells following 48-hour exposure to 500 µg/mL of *A. glabrata* extract. A. Treated AGS cells exhibit granular and vacuolated cytoplasm along with reduced adhesion to the substrate, compared to B. untreated AGS cells. C. Treated HUGU cells and D. control HUGU cells show no significant morphological differences.

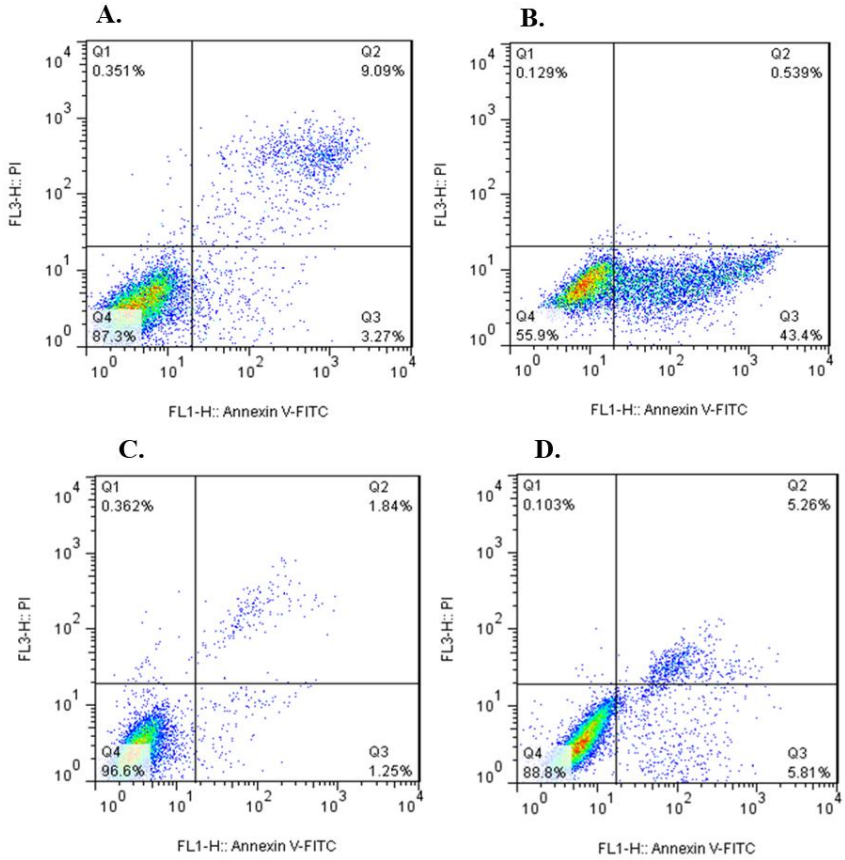

Figure 4. Flow cytometric analysis of apoptosis in AGS and HUGU cells following 48-hour treatment with *A. glabrata*. A. Untreated AGS cells (control). B. AGS cells treated with 500 µg/mL of the extract. C. Untreated HUGU cells (control). D. HUGU cells treated with 500 µg/mL of the extract.

Quantitative Gene Expression Analysis by Real-time PCR

To investigate the molecular mechanisms underlying the anticancer effects of *A. glabrata*, the expression levels of key genes involved in apoptosis and cell proliferation, including *HER2*, *HSP90α*, *P53*, and *Caspase-3*, were evaluated using real-time PCR in AGS and HUGU cells. As shown in Figure 5A–D, treatment with 500 µg/mL of *A. glabrata* extract for 48 hours resulted in a significant downregulation of *HER2* expression in AGS cells ($p<0.001$), suggesting a potential suppression of oncogenic signaling pathways. Conversely, the expression of *HSP90α*, *P53* ($p<0.05$), and *Caspase-3* ($p<0.001$) was significantly upregulated in the treated AGS cells compared with the untreated control group, indicating the activation of apoptotic and stress-response pathways. In HUGU cells, treatment with the same concentration of extract resulted in a significant increase in *Caspase-3* expression ($p<0.01$) compared with controls (Figure 5D). In contrast, the expression levels of *HER2*, *HSP90α*, and *P53* (Figure 5A–C) remained unchanged, suggesting selective activation of apoptotic mechanisms in cancer cells without notable effects on normal cells.

These results collectively indicate that *A. glabrata* extract modulates gene expression in a manner that favors apoptosis and tumor suppression in gastric cancer cells, with minimal impact on normal cells.

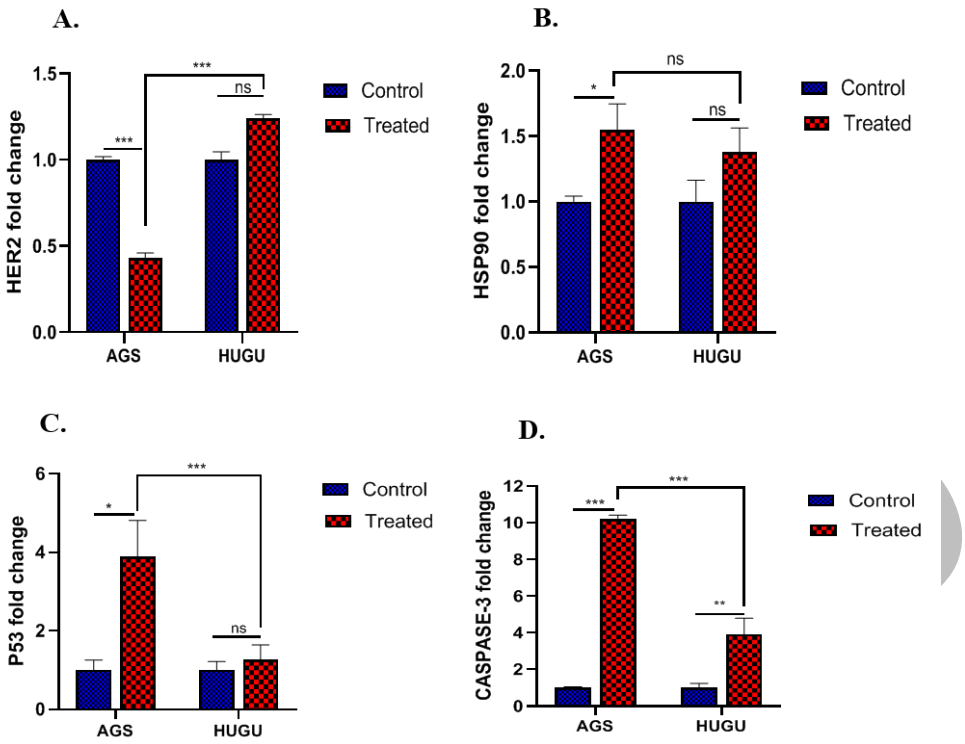

Figure 5. The effect of *A. glabrata* extract on mRNA expression levels of A. *HER2*, B. *HSP90α*, C. *P53*, and D. *Caspase-3* genes in AGS and HUGU cell lines. AGS and HUGU cells were treated with 500 µg/mL of *A. glabrata* methanolic extract for 48 hours. Gene expression levels were assessed by real-time PCR and normalized to *GAPDH*. In AGS cells, treatment significantly downregulated *HER2* (p<0.001), while significantly upregulated *HSP90α*, *P53* (p<0.05), and *Caspase-3* (p<0.001) compared to the untreated control. In HUGU cells, only *Caspase-3* expression was significantly increased (p<0.01), with no significant changes observed in *HER2*, *HSP90α*, or *P53* expression. Data are presented as mean±SD from three independent experiments performed in triplicate. *p<0.05, **p<0.01, and ***p<0.001 vs control.

DISCUSSION

Gastric cancer is one of the most common and deadly malignancies worldwide, and due to late diagnosis, aggressive tumor behavior, and the limitations of available treatments, it contributes significantly to global morbidity and mortality.[19] Despite advancements in surgery, chemotherapy, and targeted therapies, treatment resistance and systemic toxicity remain major clinical challenges. In this context, there has been growing attention to natural compounds and medicinal plants as complementary or alternative therapeutic options, as these compounds can impact multiple molecular pathways associated with cancer, have lower toxicity, and offer multi-target therapeutic effects.[3] This study was designed to evaluate the anticancer and antioxidant effects of the methanolic extract of *A glabrata* on AGS cells, with a particular focus on apoptosis induction and the regulation of the expression of *HER2*, *P53*, *Caspase-3*, and *HSP90α* genes.

Initially, the antioxidant activity of *A. glabrata* extract was assessed using the DPPH assay, demonstrating its effective free radical scavenging capacity and potential to reduce oxidative stress. Oxidative stress is a major contributor to DNA damage, genomic instability, and cancer initiation, especially in gastric cancer.[20] Previous studies have shown that medicinal plants rich in bioactive compounds, such as flavonoids, alkaloids, terpenes, and phenolic acids, possess strong antioxidant properties that can suppress oxidative stress and thereby inhibit the initiation and progression of cancer.[21] Additionally, several plant extracts, including olive, green tea, grapes, and turmeric, have been reported to enhance antioxidant defense mechanisms and protect cells against oxidative damage, which is closely associated with cancer development.[22]

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Accordingly, the findings of the present study are consistent with previous studies and suggest that the bioactive compounds in *A. glabrata* may reduce oxidative stress, limit cancer progression, and potentially serve as a complementary approach to conventional cancer therapies.

The cytotoxic effects of the methanolic extract of *A. glabrata* on AGS and HUGU cell lines were evaluated using the MTT assay. The extract inhibited cell proliferation in a dose-dependent manner, with a markedly greater effect on AGS cells than on normal cells. After 48 hours of exposure, the IC_{50} values were approximately 500 µg/mL for AGS cells and 1280 µg/mL for HUGU cells, indicating selective cytotoxicity toward gastric cancer cells. This selective growth inhibition is a desirable feature in cancer therapy, as conventional treatments often damage healthy cells. Consistent with previous studies, plant-derived bioactive compounds such as flavonoids and terpenes have been reported to suppress cancer cell proliferation with limited toxicity to normal cells.[23, 24] Collectively, these findings suggest that *A. glabrata* extract may represent a promising natural adjunct for gastric cancer therapy.

At the molecular level, a significant decrease in *HER2* gene expression was observed in AGS cells, while no significant change was noted in HUGU cells. *HER2* is a well-established oncogene implicated in the pathogenesis of several malignancies, including gastric and breast cancers, and its overexpression is closely associated with enhanced tumor growth, survival, and poor prognosis. Therefore, the inhibition of *HER2* expression by *A. glabrata* suggests a potential mechanism through which the extract may inhibit cancer cell proliferation and survival. Consistent with these observations, previous studies have reported similar effects for plant-derived bioactive compounds. Notably, Yan et al demonstrated that honokiol markedly downregulated *HER2* expression and inhibited the *PI3K/AKT* signaling pathway in *HER2*-positive gastric cancer cells, thereby promoting apoptotic cell death.[22] Furthermore, Gholami et al found that the methanolic extract of *Alkanna bracteosa* suppressed *HER2* expression in AGS cells and induced apoptosis.[25]

Apoptosis is a tightly regulated and essential biological process responsible for eliminating damaged or abnormal cells and maintaining tissue homeostasis. Disruption of apoptotic signaling is a hallmark of cancer, enabling uncontrolled cell survival and proliferation. *Caspase-3* is a key executioner enzyme in the apoptotic cascade, and its activation represents a critical step in the irreversible commitment of cells to programmed death.[18] In the present study, *A. glabrata* extract significantly increased *Caspase-3* activity in AGS cells, indicating the induction of apoptosis. A modest increase in *Caspase-3* activity was also observed in HUGU cells, although the effect was markedly greater in AGS cells, highlighting the preferential cytotoxicity of the extract toward cancer cells. This observation is consistent with previous studies demonstrating that plant-derived compounds, including resveratrol and curcumin, activate *Caspase-3* and promote apoptotic pathways in cancer cells.[26–29]

In parallel, treatment with *A. glabrata* extract led to a significant upregulation of *P53* gene expression in AGS cells. *P53*, widely recognized as the "guardian of the genome," plays a central role in preventing the propagation of genetically damaged cells by regulating cell cycle arrest and apoptosis.[17,30,31] Activation of *P53* is considered a crucial antitumor defense mechanism, and its increased expression supports the ability of the extract to suppress tumor growth through apoptotic signaling. These findings are in agreement with previous reports highlighting the importance of *P53* activation in inhibiting cancer progression and promoting programmed cell death.[31–33]

The present study also demonstrated that the extract of *A. glabrata* significantly increased the expression of *HSP90α* in AGS cells, whereas no significant alteration was observed in HUGU cells. *HSP90α*, a molecular chaperone protein, plays a key role in stabilizing oncogenic and stress-responsive proteins and is typically activated in response to cellular stresses such as oxidative stress, DNA damage, or exposure to cytotoxic agents.[34] The upregulation observed in AGS cells likely represents a stress-adaptive response to the cytotoxic and oxidative effects of the extract. However, the simultaneous activation of *P53* and *Caspase-3* indicates that this protective response is insufficient, ultimately leading to apoptosis. This dual effect highlights the extract's selective cytotoxicity toward gastric cancer cells, while sparing normal cells. Previous studies have shown that *HSP90* inhibition can reduce cancer cell proliferation and increase their sensitivity to anticancer agents.[34,35] The selective modulation of *HSP90α* expression in cancer cells, with no significant effect on normal cells, further supports the selective cytotoxic profile of the *A. glabrata* extract.

Flow cytometric analysis also revealed a marked increase in both early and late apoptotic cell populations in AGS cells following treatment, corroborating the molecular findings and confirming apoptosis as a primary mechanism underlying the anticancer activity of the extract. Collectively, the antioxidant capacity, selective cytotoxicity, and modulation of key molecular pathways suggest that *A. glabrata* extract may exert effects on several cellular pathways, which could provide potential advantages over single-target therapeutic agents. Despite these encouraging findings, several limitations of the present study should be acknowledged. The study was conducted exclusively under in vitro conditions using a limited number of cell lines, which may not fully recapitulate the complexity of the in vivo tumor microenvironment. Therefore, in vivo studies are warranted to assess the efficacy and safety of the extract. Moreover, the use of a crude methanolic extract precludes identification of the specific bioactive constituents responsible for the observed effects, highlighting the need for further phytochemical isolation and characterization. Additionally, molecular analyses were limited to the mRNA level, and validation at the protein level is required. Finally, potential interactions between *A. glabrata* extract and conventional chemotherapeutic agents were not evaluated, and future combination studies may help clarify its role as a complementary therapeutic strategy.

The results of this study demonstrate that the methanolic extract of *A. glabrata* exhibits selective anticancer and significant antioxidant activities on AGS gastric cancer cells. In AGS gastric adenocarcinoma cells, the extract inhibited proliferation in a dose-dependent manner, increased the expression of pro-apoptotic genes such as *Caspase-3* and *P53*, and decreased *HER2* expression. By inducing apoptosis, it effectively reduced cancer cell viability. These findings suggest that *A. glabrata* may influence several cellular pathways, including antioxidant activity, *HER2* inhibition, and apoptosis induction. However, as these results are based solely on in vitro experiments, any suggestion regarding clinical applicability should be considered preliminary. Further studies, including in vivo animal models and pharmacokinetic analyses, are required to evaluate the efficacy, safety, and active compounds of the extract. Such studies will help clarify the potential role of *A. glabrata* as a complementary or adjunctive treatment alongside current chemotherapy regimens.

STATEMENT OF ETHICS

The present study was conducted using commercially available cell lines obtained from the Iranian Biological Resources Center (IBRC). As the study did not involve human participants or animals, and utilized only established cell lines, formal approval from an institutional ethics committee was not required at the time of the study. However, all experiments were performed in accordance with the relevant guidelines and regulations for the handling and use of biological materials.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

AI ASSISTANCE DISCLOSURE

The authors used Grammarly (https://www.grammarly.com) for language polishing and grammar checking. No AI tools were used for data analysis, figure generation, or scientific writing.

REFERENCES

1. Thrift AP, El-Serag HB. Burden of gastric cancer. Clin Gastroenterol Hepatol. 2020;18(3):534–542.
2. Thrumurthy SG, Chaudry MA, Hochhauser D, Mughal M. The diagnosis and management of gastric cancer. BMJ. 2013;347:f6367.

3. Machlowska J, Baj J, Sitarz M, et al. Gastric cancer: Epidemiology, risk factors, classification, genomic characteristics and treatment strategies. Int J Mol Sci. 2020;21(11):4012.
4. Vasan N, Baselga J, Hyman DM. A view on drug resistance in cancer. Nature. 2019;575(7782):299–309.
5. Ali M, Wani SUD, Salahuddin M, et al. Recent advance of herbal medicines in cancer—a molecular approach. Heliyon. 2023;9(2).
6. Gezici S, Şekeroğlu N. Current perspectives in the application of medicinal plants against cancer: Novel therapeutic agents. Anticancer Agents Med Chem. 2019;19(1):101–111.
7. Cuevas-Cianca SI, Romero-Castillo C, Gálvez-Romero JL, Juárez ZN, Hernández LR. Antioxidant and anti-inflammatory compounds from edible plants with anti-cancer activity and their potential use as drugs. Molecules. 2023;28(3):1488.
8. Akbari B, Baghaei-Yazdi N, Bahmaie M, Mahdavi Abhari F. The role of plant-derived natural antioxidants in reduction of oxidative stress. Biofactors. 2022;48(3):611–633.
9. Escobar García P, Pakravan M, Schönswetter P, Aguilar JF, Schneeweiss GM. Phylogenetic relationships in the species-rich Irano-Turanian genus Alcea (Malvaceae). Taxon. 2012;61(2):324–332.
10. Azab A. Alcea: Traditional medicine, current research and future opportunities. Eur Chem Bull. 2016;5(12):505–514.
11. Pirmohammadi Y, Asnaashari S, Nazemiyeh H, Hamedeyazdan S. Bioactivity assays and phytochemical analysis upon Alcea glabrata; focusing on xanthine oxidase inhibitory and antimalarial properties. Toxicon. 2023;229:107140.
12. Abutaha N, Wadaan MA, Alghamdi RA, Al-Mekhlafi FA. Potential anticancer activity of Alcea rosea and molecular docking of selected target proteins in liver cancer cells. Int J Morphol. 2024;42(6).
13. Grillo F, Fassan M, Sarocchi F, Fiocca R, Mastracci L. HER2 heterogeneity in gastric/gastroesophageal cancers: From benchside to practice. World J Gastroenterol. 2016;22(26):5879–5887.
14. Abrahao-Machado LF, Scapulatempo-Neto C. HER2 testing in gastric cancer: An update. World J Gastroenterol. 2016;22(19):4619–4625.
15. Liu B, Qian D. Hsp90α and cell death in cancers: A review. Discov Oncol. 2024;15(1):151.
16. Birbo B, Madu EE, Madu CO, Jain A, Lu Y. Role of HSP90 in cancer. Int J Mol Sci. 2021;22(19):10317.
17. Ozaki T, Nakagawara A. Role of p53 in cell death and human cancers. Cancers (Basel). 2011;3(1):994–1013.
18. Elmore S. Apoptosis: A review of programmed cell death. Toxicol Pathol. 2007;35(4):495–516.
19. Park JY, von Karsa L, Herrero R. Prevention strategies for gastric cancer: A global perspective. Clin Endosc. 2014;47(6):478–489.
20. Chen Z, Fan J, Chen X, Yang K, Wang K. Oxidative stress and redox signaling in gastric cancer: From mechanisms to therapeutic implications. Antioxidants (Basel). 2025;14(3):258.
21. Njoya EM. Medicinal plants, antioxidant potential, and cancer. In: Cancer. Amsterdam: Elsevier; 2021. p. 349–357.
22. Rodríguez-Yoldi MJ. Anti-inflammatory and antioxidant properties of plant extracts. Basel: MDPI; 2021. p. 921.
23. Pandey P, Lakhanpal S, Mahmood D, et al. An updated review summarizing the anticancer potential of flavonoids via targeting NF-κB pathway. Front Pharmacol. 2025;15:1513422.
24. Ding Y, Yu Y. Therapeutic potential of flavonoids in gastrointestinal cancer: Focus on signaling pathways and improvement strategies. Mol Med Rep. 2025;31(4):109.
25. Gholami M, Elyasigorji Z, Amoli AD, Farzaneh P. Effects of Alkanna bracteosa extract on the expression level of HSP90α and HER2 genes in human gastric cancer cell line. Adv Tradit Med. 2023;23(3):877–886.
26. Zhu L, Han MB, Gao Y, et al. Curcumin triggers apoptosis via upregulation of Bax/Bcl-2 ratio and caspase activation in SW872 human adipocytes. Mol Med Rep. 2015;12(1):1151–1156.
27. Zamanian MY, Turkadze KA, Sadeghi Ivraghi M, Golmohammadi M, Sharifi M, Keshtpour Amlashi Z. Curcumin and resveratrol as dual modulators of the STAT3 pathway in lung cancer: A comprehensive review. Food Sci Nutr. 2025;13(9):e70829.
28. Reuter S, Eifes S, Dicato M, Aggarwal BB, Diederich M. Modulation of anti-apoptotic and survival pathways by curcumin as a strategy to induce apoptosis in cancer cells. Biochem Pharmacol. 2008;76(11):1340–1351.
29. Raviv T, Shteinfer-Kuzmine A, Moyal MM, Shoshan-Barmatz V. Resveratrol's pro-apoptotic effects in cancer are mediated through the interaction and oligomerization of the mitochondrial VDAC1. Int J Mol Sci. 2025;26(9):3963.
30. Wang H, Guo M, Wei H, Chen Y. Targeting p53 pathways: Mechanisms, structures and advances in therapy. Signal Transduct Target Ther. 2023;8(1):92.

31. Borrero LJH, El-Deiry WS. Tumor suppressor p53: Biology, signaling pathways, and therapeutic targeting. Biochim Biophys Acta Rev Cancer. 2021;1876(1):188556.
32. Athar M, Elmets CA, Kopelovich L. Pharmacological activation of p53 in cancer cells. Curr Pharm Des. 2011;17(6):631–639.
33. Peng Y, Bai J, Li W, Su Z, Cheng X. Advancements in p53-based anti-tumor gene therapy research. Molecules. 2024;29(22):5315.
34. Jhaveri K, Modi S. HSP90 inhibitors for cancer therapy and overcoming drug resistance. Adv Pharmacol. 2012;65:471–517.
35. Li ZN, Luo Y. HSP90 inhibitors and cancer: Prospects for use in targeted therapies. Oncol Rep. 2022;49(1):6.