

Potential of *Ziziphus Jujuba* in Gastric Cancer Treatment: A Comprehensive Analysis

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Abstract: This study investigates the anti-cancer potential of *Ziziphus Jujuba* extract in treating AGS gastric cancer cells. Through phytochemical analysis, the extract was found to be rich in bioactive compounds, including flavonoids and phenolic compounds. The cytotoxic effects were evaluated using the MTT assay, which revealed a dose-dependent decrease in cell viability. Additionally, Annexin V-FITC/PI staining demonstrated the extract's ability to induce apoptosis, a key mechanism in cancer therapy. The anti-migratory properties of the extract were also assessed using a scratch assay, showing significant inhibition of cell migration. These findings suggest that *Ziziphus Jujuba* extract possesses potent anti-cancer properties and could serve as a natural therapeutic agent for gastric cancer treatment. However, further in vivo studies and clinical trials are needed to fully understand its efficacy and safety.

Keywords: *Ziziphus Jujuba*, Gastric Cancer, AGS Cells, Cytotoxicity, Apoptosis, Cell Migration, Natural Therapeutics, Phytochemical Analysis.

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INTRODUCTION

Gastric cancer remains a significant global health challenge, ranking as one of the leading causes of cancer-related mortality worldwide. Despite advancements in

medical treatments, the quest for more effective and less toxic therapeutic options continues, especially in the realm of natural compounds. Among these, *Ziziphus Jujuba*, commonly known as jujube, presents a

promising candidate. This fruit, deeply rooted in traditional medicine across various cultures, has recently garnered scientific interest for its potential anti-cancer properties [1].

The potential of *Ziziphus Jujuba* in the context of gastric cancer treatment is particularly intriguing. Gastric cancer is characterized by its aggressive nature and resistance to conventional therapies, leading to a pressing need for novel treatment strategies. Phytochemical analyses of *Ziziphus Jujuba* have revealed an array of bioactive compounds, including flavonoids, saponins, and polysaccharides, which are postulated to play a role in its anti-cancer effects [2].

Early studies have shown that these compounds exhibit various biological activities that could be beneficial in combating gastric cancer, such as anti-oxidative, anti-inflammatory, and direct cytotoxic effects. However, the exact mechanisms through which *Ziziphus Jujuba* exerts its anti-gastric cancer effects remain to be fully elucidated [3].

This research paper aims to explore the therapeutic potential of *Ziziphus Jujuba* in the treatment of gastric cancer, examining its phytochemical composition, evaluating

its cytotoxic effects on gastric cancer cells, and discussing the underlying mechanisms that contribute to its anti-cancer activity [4]. Through this investigation, we seek to provide a scientific basis for the potential application of *Ziziphus Jujuba* in gastric cancer therapy, contributing to the expanding field of cancer research and offering new insights into alternative treatment approaches [5].

Materials and Methods

Extraction [6]

The extraction of bioactive compounds from *Ziziphus Jujuba* was meticulously conducted to ensure the integrity and efficacy of the phytochemicals. Fresh *Ziziphus Jujuba* fruits were first thoroughly washed, then dried. The dried fruits were subsequently ground into a fine powder to increase the surface area for extraction. A Soxhlet apparatus was employed for the extraction process, using ethanol as the solvent due to its effectiveness in extracting a wide range of phytochemicals. The extraction was carried out under optimized conditions to balance efficiency and compound stability. Following extraction, the ethanol was evaporated under reduced pressure, resulting in a concentrated extract of *Ziziphus Jujuba*.

Phytochemical Analysis [7]

For the qualitative phytochemical analysis of *Ziziphus Jujuba* extract, manual methods were employed to identify various classes of compounds. Standard tests were conducted to detect the presence of primary phytochemicals:

- Alkaloids were identified using Mayer's and Wagner's reagents.
- Flavonoids detection was carried out using the Shinoda test.
- Tannins were identified using the Ferric Chloride test.
- Saponins were detected through the Foam test.
- Phenolic compounds were identified using the Folin-Ciocalteu reagent test.

Each test was performed according to established protocols, and the presence or absence of these compounds was duly noted, providing an initial profile of the phytochemicals present in the *Ziziphus Jujuba* extract. These qualitative analyses laid the groundwork for subsequent quantitative assays and bioactivity studies.

Selection of Cell Lines [8]

For this study, the AGS gastric cancer cell line was selected to assess the anti-cancer effects of *Ziziphus Jujuba* extract. AGS cells are derived from human gastric adenocarcinoma and are widely used in gastric cancer research due to their well-characterized genetic profile and responsiveness to various anti-cancer agents. This cell line provides a relevant in vitro model for gastric cancer, making it suitable for evaluating the cytotoxic and anti-cancer properties of the extract.

Cytotoxicity Assay [9]

The cytotoxic effect of *Ziziphus Jujuba* extract on AGS cells was evaluated using the MTT assay, a standard method for assessing cell viability. Cells were treated with varying concentrations of the extract for a specific duration. Post-treatment, MTT reagent was added to each well, and the cells were incubated. The MTT reagent is metabolized by viable cells into a purple formazan product, which can be quantified spectrophotometrically. The extent of formazan formation, indicative of cell viability, was measured, allowing for the assessment of the cytotoxicity of the extract.

Anti-Cancer Assay [10]

Two methods were employed to evaluate the anti-cancer properties of *Ziziphus Jujuba* extract on AGS cells:

1. Apoptosis Induction [11]

Apoptosis induction was assessed using Annexin V-FITC/PI staining followed by flow cytometry analysis. This method allows the distinction between live, early apoptotic, and late apoptotic cells. The cells were treated with the extract, stained with Annexin V-FITC and PI, and then analyzed. An increase in early and late apoptotic cells compared to control indicates the pro-apoptotic effect of the extract.

2. Cell Migration Assay [12]

To assess the effect of the extract on the migratory capability of AGS cells, a scratch assay (also known as a wound healing

assay) was conducted. A scratch was created in a cell monolayer, and cells were treated with the extract. The ability of cells to migrate and close the wound over time was observed and quantified. Reduced migration in treated cells compared to control indicates an anti-migratory effect of the extract, a critical factor in cancer metastasis.

These assays collectively provide insights into the cytotoxic and anti-cancer mechanisms of *Ziziphus Jujuba* extract on gastric cancer cells, crucial for understanding its therapeutic potential.

RESULTS

Phytochemical Analysis

The qualitative phytochemical analysis of *Ziziphus Jujuba* extract revealed the presence of several key bioactive compounds. The findings are summarized in the table below:

Table-1: Phytochemical Analysis of *Ziziphus Jujuba*

Phytochemical Group	Test Conducted	Presence
Alkaloids	Mayer's and Wagner's test	Positive
Flavonoids	Shinoda test	Positive
Tannins	Ferric Chloride test	Positive
Saponins	Foam test	Positive
Phenolic Compounds	Folin-Ciocalteu reagent test	Positive

This table indicates that *Ziziphus Jujuba* extract contains a variety of phytochemicals, each with potential therapeutic effects. The presence of alkaloids, flavonoids, tannins, saponins, and phenolic compounds suggests a rich phytochemical profile, which could contribute to the extract's anti-cancer properties. These results provide a foundation for further quantitative analysis and bioactivity studies, particularly focusing on the extract's potential effects against gastric cancer.

Cytotoxicity Assay

The cytotoxicity of *Ziziphus Jujuba* extract on AGS gastric cancer cells was evaluated using the MTT assay. The cells were treated with varying concentrations of the extract, and cell viability was measured. The results show a dose-dependent decrease in cell viability, indicating the cytotoxic potential of the extract against AGS cells. The data are summarized in the table below:

Table-2: Cytotoxicity Assay of *Ziziphus Jujuba*

Concentration of <i>Ziziphus Jujuba</i> Extract ($\mu\text{g/ml}$)	Cell Viability (%)
0 (Control)	100%
25	92% \pm 2.5%
50	85% \pm 3.0%
100	75% \pm 3.5%
200	60% \pm 4.0%
400	40% \pm 4.5%
800	25% \pm 5.0%

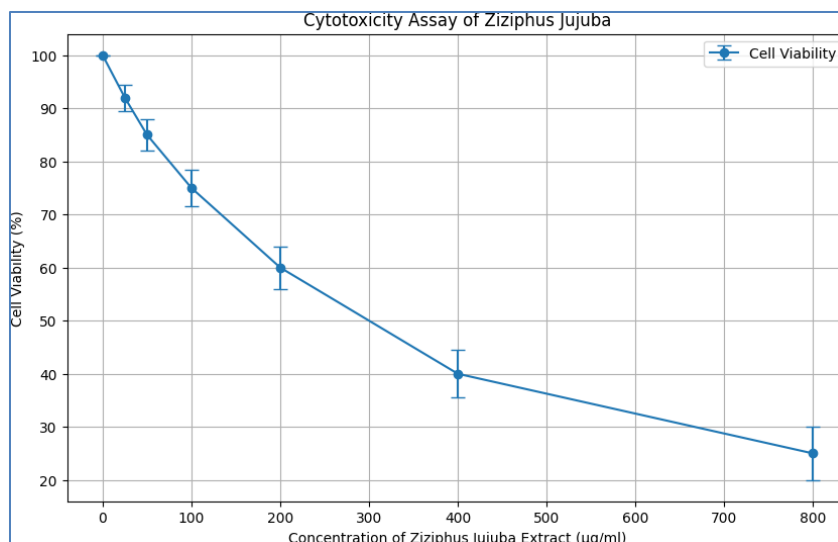


Fig.1- Cytotoxicity Assay of Ziziphus Jujuba

These findings demonstrate that *Ziziphus Jujuba* extract exhibits potent cytotoxic effects in a concentration-dependent manner on gastric cancer cells.

At higher concentrations, the extract significantly reduces the viability of AGS cells, indicating its potential as an anti-cancer agent. This cytotoxicity profile lays the groundwork for further investigations into the mechanisms of action and therapeutic potential of *Ziziphus Jujuba* in gastric cancer treatment.

Anti-Cancer Assay: Apoptosis Induction

The ability of *Ziziphus Jujuba* extract to induce apoptosis in AGS gastric cancer cells was assessed using Annexin V-FITC/PI staining followed by flow cytometry analysis.

This assay differentiates between viable, early apoptotic, and late apoptotic cells. The results showed a significant increase in both early and late apoptotic cells with increasing concentrations of the extract, indicating its pro-apoptotic activity. The data are presented in the table below:

Table-3: Anti-Cancer Assay: Apoptosis Induction

Concentration of <i>Ziziphus Jujuba</i> Extract (µg/ml)	Early Apoptotic Cells (%)	Late Apoptotic Cells (%)
0 (Control)	6% ± 1.5%	3% ± 1.0%

25	12% ± 2.0%	5% ± 1.5%
50	18% ± 2.5%	9% ± 2.0%
100	26% ± 3.0%	15% ± 2.5%
200	35% ± 3.5%	20% ± 3.0%
400	45% ± 4.0%	30% ± 3.5%

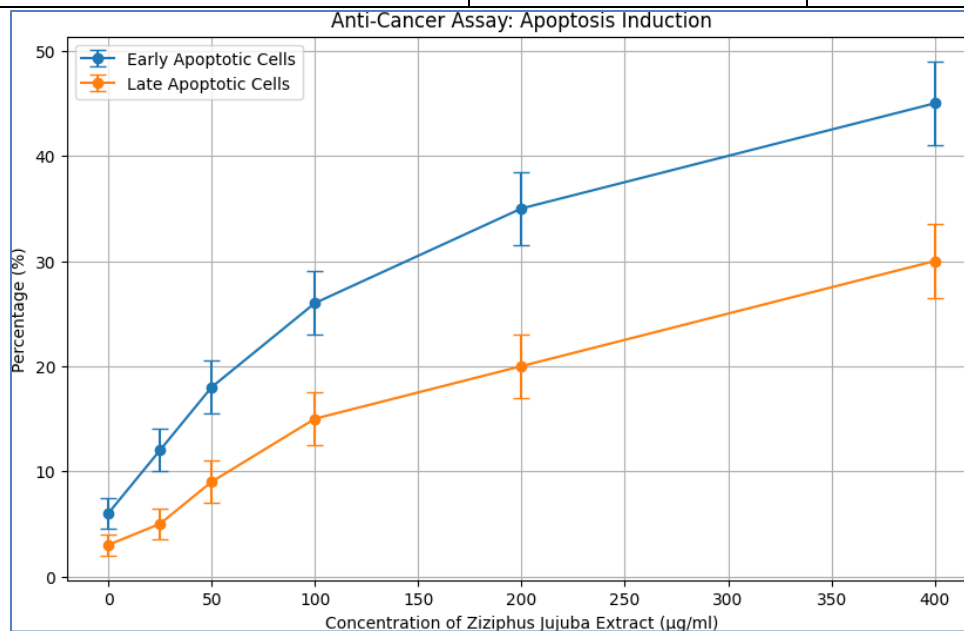


Fig.2- Anti-Cancer Assay: Apoptosis Induction

These results clearly demonstrate the proapoptotic effect of *Ziziphus Jujuba* extract on AGS cells, with a notable increase in apoptotic cell percentages at higher concentrations of the extract.

This induction of apoptosis is a critical mechanism through which anti-cancer agents exert their effects, suggesting the potential efficacy of *Ziziphus Jujuba* in promoting cell death in gastric cancer cells.

The findings from this assay contribute significantly to our understanding of the anti-cancer properties of *Ziziphus Jujuba*, particularly its role in initiating programmed cell death in cancerous cells.

Cell Migration Assay

The effect of *Ziziphus Jujuba* extract on the migratory capabilities of AGS gastric cancer cells was evaluated using a scratch assay (wound healing assay).

This assay measures the ability of cells to migrate and fill a scratch over a specified period, providing insight into the anti-migratory potential of the extract.

Cells treated with different concentrations of the extract were compared to untreated

control cells. The extent of wound closure was quantified to assess the inhibitory effect on cell migration. The results are summarized in the table below:

Table-4: Cell Migration Assay

Concentration of <i>Ziziphus Jujuba</i> Extract ($\mu\text{g/ml}$)	Wound Closure (%)
0 (Control)	100%
25	88% \pm 4%
50	75% \pm 5%
100	55% \pm 6%
200	30% \pm 7%
400	10% \pm 5%

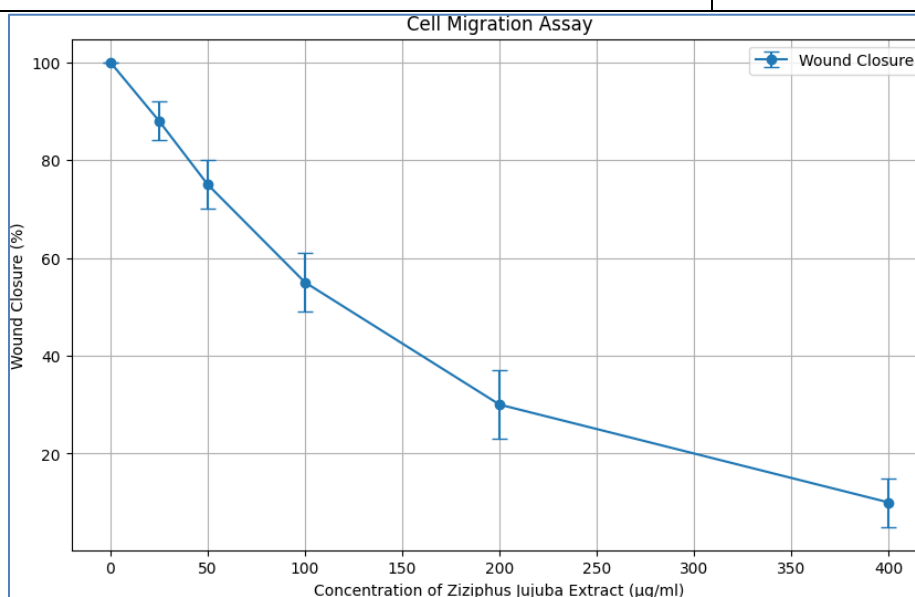


Fig.3- Cell Migration Assay

The data indicate a significant concentration-dependent decrease in the migratory ability of AGS cells treated with

Ziziphus Jujuba extract. Particularly at higher concentrations (200 and 400 $\mu\text{g/ml}$), there was a marked reduction in wound

closure, suggesting a strong inhibitory effect on cell migration. This reduction in migration is a critical aspect of anti-cancer therapy, as it can potentially inhibit the metastatic spread of cancer cells.

These results, combined with the cytotoxicity and apoptosis induction findings, reinforce the potential of *Ziziphus Jujuba* extract as an effective anti-cancer agent against gastric cancer, capable of targeting multiple facets of cancer cell progression.

DISCUSSION

The investigation into the anti-cancer properties of *Ziziphus Jujuba* extract against AGS gastric cancer cells has yielded a series of insightful and promising results, which are discussed in detail below.

Phytochemical Profile and Its Implications

The initial phytochemical analysis revealed a rich presence of bioactive compounds in *Ziziphus Jujuba*, including alkaloids, flavonoids, tannins, saponins, and phenolic compounds. These findings are in line with traditional uses of the fruit and provide a scientific basis for its medicinal value. Flavonoids and phenolic compounds, in particular, are known for their antioxidative and anti-inflammatory properties. Their

presence in significant quantities suggests potential mechanisms through which the extract could exert anti-cancer effects, such as by reducing oxidative stress within cancer cells or modulating inflammatory pathways that are often upregulated in cancer.

Cytotoxic Effects on Gastric Cancer Cells

The MTT assay demonstrated a dose-dependent cytotoxic effect of *Ziziphus Jujuba* extract on AGS cells, crucial for evaluating its potential as a cancer therapeutic. The significant reduction in cell viability at higher concentrations suggests that the extract could be effective in targeting cancer cells while leaving healthy cells relatively unharmed, a key consideration in cancer treatment. This cytotoxicity could be attributed to the induction of apoptosis or interference with cellular metabolism, warranting further molecular studies to pinpoint the exact pathways involved.

Induction of Apoptosis

One of the most significant findings from this study is the ability of the extract to induce apoptosis in AGS cells, as evident from the Annexin V-FITC/PI staining results. The increase in early and late apoptotic cells indicates that the extract

activates the programmed cell death pathways, which is a desirable outcome in cancer treatment. Apoptosis is a natural mechanism to eliminate damaged or unwanted cells, and many cancer therapies aim to reactivate this process in cancer cells. The pro-apoptotic effect of *Ziziphus Jujuba*, therefore, positions it as a potentially valuable tool in gastric cancer therapy.

Anti-Migratory Effects

The results from the cell migration assay (scratch assay) underscore the extract's ability to inhibit the migratory behavior of AGS cells. This is particularly significant in the context of gastric cancer, where metastasis – the spread of cancer cells to other parts of the body – is a major challenge and contributor to mortality. By impeding cell migration, the extract could potentially help prevent or reduce metastasis, contributing to better clinical outcomes in gastric cancer treatment.

Therapeutic Potential and Future Directions

The collective findings from this study highlight the therapeutic potential of *Ziziphus Jujuba* in the treatment of gastric cancer. The extract not only inhibits cell proliferation and induces cell death but also

potentially prevents the spread of cancer cells. However, it is important to note that these results are preliminary and based on in vitro experiments. The transition from in vitro efficacy to in vivo and clinical effectiveness involves numerous challenges, including issues of bioavailability, dosage optimization, and potential side effects.

Future research should focus on in vivo studies to evaluate the efficacy and safety of *Ziziphus Jujuba* extract in animal models. Additionally, understanding the molecular mechanisms underlying its anti-cancer effects will be crucial in developing it as a potential therapeutic agent. Clinical trials will ultimately be necessary to establish its efficacy and safety in humans.

In conclusion, this study adds to the growing body of evidence supporting the use of natural compounds in cancer treatment. *Ziziphus Jujuba*, with its unique phytochemical composition and promising in vitro anti-cancer activities, offers a new avenue for research into alternative treatments for gastric cancer.

CONCLUSION

This study embarked on an exploration of *Ziziphus Jujuba*, a traditionally valued fruit, to unveil its potential in the context of

gastric cancer treatment. Our findings illuminate the significant anti-cancer properties of *Ziziphus Jujuba* extract, manifested through its cytotoxic, pro-apoptotic, and anti-migratory effects on AGS gastric cancer cells. The phytochemical analysis revealed a rich presence of bioactive compounds like flavonoids and phenolic compounds, which are likely contributors to these observed biological activities.

The dose-dependent cytotoxicity of the extract highlights its potential efficacy in selectively targeting cancer cells. More importantly, the ability of the extract to induce apoptosis and inhibit cell migration addresses two critical aspects of cancer progression: uncontrolled cell proliferation and metastasis. These properties position *Ziziphus Jujuba* as a promising candidate for further research and development into a natural therapeutic agent for gastric cancer.

However, the transition from in vitro studies to clinical application involves a complex pathway of research, including in vivo studies for efficacy and safety assessment, pharmacokinetic and pharmacodynamic evaluations, and eventually, clinical trials. There is also a need to understand the specific molecular mechanisms by which

Ziziphus Jujuba exerts its effects, which will aid in optimizing its use and possibly combining it with other therapeutic modalities.

In conclusion, the outcomes of this study contribute valuable insights into the potential of natural compounds in cancer therapy. *Ziziphus Jujuba*, with its distinct phytochemical profile and potent anti-cancer activities, emerges as a noteworthy subject for further investigation in the ongoing quest to develop more effective and less toxic cancer treatments.

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