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Pharmacological Evaluation of Syzygium Cumini Aqueous Seed Extract with Special Reference to Anticancer and Anti Inflammatory Mechanisms in Human Carcinoma Cell Lines

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Abstract
The medicinal relevance of Syzygium cumini (L.) Skeels as a widely used drug in traditional medicine due to its varied therapeutic benefit has been underutilized, but its anticancer and anti-inflammatory effects are under-studied. This paper has explored the pharmacological impact of Syzygium cumini aqueous seed extract on human carcinoma cell lines with further focus on cytotoxicity, induction of apoptosis and alteration of cytokines. The cells that were cultured and exposed to different concentrations of extract were the HeLa (cervical carcinoma), MCF-7 (breast carcinoma) and normal fibroblast cells. Cytotoxicity was induced with the MTT assay, apoptosis with annexin V/PI staining, and anti-inflammatory effects by ELISA determination of IL-6 and TNF- 953 assays of LPS-stimulated cells. The extract exhibited the dose-dependent cytotoxicity of carcinoma cells and spared fibroblasts with the cell viability decreasing to approximately 2831 at 200 µg /mL. At higher doses the apoptotic index was found to increase dramatically reaching more than 50% which proved that the main mechanism of cytotoxicity was apoptosis. In addition, there was a significant inhibition of IL-6 and TNF-alpha levels, and this was an indication of high-grade anti-inflammation. The statistical validation conducted using ANOVA and Tukey test proved significant difference of these findings ($p < 0.05$). In sum, the research results point to the dual anticancer and anti-inflammatory nature of Syzygium cumini aqueous seed extract, as well as further investigation as a natural therapeutic agent in cancer treatment.

Keywords: *Syzygium cumini, carcinoma cell lines, apoptosis, cytotoxicity, anti-inflammatory activity, cytokine modulation*

I. INTRODUCTION

Cancer has over the years been one of the greatest causes of morbidity and mortality globally and even with the modern treatment methods that have been developed there are still problems like drug resistance, side effects and high cost of treatment that are limiting the effective management of cancer. As a result, the trend has been the increased interest in the natural products as possible sources of safe and low-cost anticancer agents. Traditional systems of healthcare have long been reliant on medicinal plants, which provide bioactive compounds with a wide range of pharmacological benefits. Syzygium cumini (popularly called Jamun or black plum) is one of these and has been extensively employed in ethnomedicine with regard to antidiabetic, antioxidant, anti-inflammatory and antimicrobial properties. The recent studies have also indicated that it may have anticancer effects, especially because of the existence of polyphenols, flavonoids, tannins, and other phytochemicals. Nevertheless, little has been done to completely explore how it can contend with cancer cell survival and tumor-related inflammation. The present research was hence done to assess the anticancer and anti-inflammatory effects of Syzygium cumini aqueous seed extract in human carcinoma cell lines in order to give scientific validation to its use as a pharmaceutical agent in cancer management.

1.1. Background of the Study

The development of cancer is a complicated and progressive process that includes genetic mutations, uncontrolled proliferation of individual cells, and apoptosis escape that are commonly accompanied by chronic inflammation that facilitates the growth and progression of tumors. The traditional therapies like chemotherapy and radiotherapy, though effective, have often been linked to serious side effects and lack of specificity to the cancerous cells hence the need of finding safer and more targeted options. Syzygium cumini (L.) Skeels, also referred to as Jamun, has been conventionally used in Ayurveda and folk medicine to treat diabetes, gastrointestinal diseases, and inflammatory diseases. Its seeds, especially, contain abundant amounts of phytochemicals such as flavonoids, polyphenols, tannins, and even terpenoids, which possess strong antioxidant, anti-inflammatory, as

well as anticancer properties. It has been observed in previous studies that S. cumini extracts exhibit cytotoxicity against different cancer cell lines and regulate inflammatory cytokines, which implies that the extract has a two-fold action mechanism, making it a potential therapeutic agent. Nonetheless, assessed methodically on the effectiveness of its action on human carcinoma cells and the explanation of the mechanism of action are still lacking, so its application as a natural candidate in the treatment of the cancer is still in need of research.

1.2. Therapeutic Potential of Syzygium cumini Seeds

Syzygium cumini seeds are widely used in traditional medicine for their purported health benefits. These benefits are believed to be due to the presence of bioactive substances such as tannins, terpenoids, polyphenols, and flavonoids. Research has shown that these phytochemicals can fight cancer, inflammation, diabetes, bacteria, and viruses. Specifically, the seeds have shown selective cytotoxicity to many cancer cell lines and can induce apoptosis and prevent cell proliferation whilst sparing normal cells. Taken together these properties make Syzygium cumini seeds a promising natural source of therapy with dual anticancer and anti-inflammatory effects and therefore warrant further development of their pharmacological uses.

1.3. Research objectives

- To evaluate the cytotoxic effects of *Syzygium cumini* aqueous seed extract on human carcinoma cell lines (HeLa and MCF-7) and compare them with normal fibroblast cells.
- To investigate the ability of *Syzygium cumini* aqueous seed extract to induce apoptosis in carcinoma cells.
- To assess the anti-inflammatory activity of the extract.
- To analyze and validate the statistical significance of the extract's anticancer and anti-inflammatory effects through appropriate biostatistical tools.

II. LITERATURE REVIEW

Suhail et al. (2025) assessed the anticancer activity of extracts of Syzygium cumini and Syzygium jambolanum against the MCF-7 breast carcinoma cells in vitro. They showed that both extracts had dose-dependent cytotoxic effects, and their viability of the cells was significantly decreased. Apoptosis was also reported to occur in the extracts, which emphasize the capability of these extracts to cause programmed cell death and not nonspecific necrosis. This was evidenced by morphological alteration in the treated cells indicating the activation of apoptotic signaling. The results established that Syzygium cumini had bioactive phytochemicals that have selective cytotoxicity to cancer cells, which supports therapeutic potential of plant-derived agents in the management of breast cancer.

Qamar et al. (2021) researched on in vitro and in vivo anti-inflammatory effects of Syzygium cumini fruit extracts. They found that they had a potent inhibitory effect on pro-inflammatory cytokines, including IL-6 and TNF- 2 in cell culture and animal models. The extract was found to regulate inflammatory response by lowering the production of cytokines and inhibiting their oxidative stress markers. These researchers came to the conclusion that the anti-inflammatory activity could be explained by the abundance of polyphenols, flavonoids, and anthocyanins in the fruit. These results were valuable indicators that Syzygium cumini had the potential to curb the pathological processes mediated by inflammation and provided scientific support to the ethnomedicinal use of Syzygium cumini in the treatment of inflammatory diseases.

Abdulrahman and Hama (2023) performed a systematic review of the anticancer activity of the genus Syzygium. Their review summarized findings of several in vitro and in vivo experiments, wherein extracts of several species of Syzygium, such as Syzygium cumini, showed a wide spectrum of anticancer effects. The anticancer activity was commonly mediated by the induction of apoptosis, cell cycle arrest and prevention of tumor-related inflammation. They have found that flavonoids, tannins, and ellagic acid phytochemicals were important in these mechanisms. The potential of Syzygium extracts as safer alternatives or adjuncts to conventional chemotherapy was also highlighted in the review, but the authors provided that more mechanistic research and clinical trials are needed to determine the therapeutic effectiveness.

Pal et al. (2024) studied the antiproliferative, morphology, and chemistry of black plum (Syzygium cumini) seeds. Their article pointed out that the seeds had a strong composition of bioactive compounds, which had a major antiproliferative effect on cancerous cells. It was proved that seed extracts inhibited the growth of cancer cells in a dose-dependent fashion and recommended that the mechanism involved apoptosis. In addition, the authors indicated that the excellent concentration of phytochemicals in seeds gave a robust pharmacological foundation of their therapeutic application. The research was significant especially because it strengthened the usefulness of Syria cumini seeds as opposed to other plant extracts as a potential source of anticancerous agents.

Rayzah et al. (2023) studied the anticancer effects of green-synthesized titanium dioxide nanoparticles prepared using fruit extract of Syzygium cumini on hepatic cancer cells. Their analysis showed that the nanoparticles had a caspase dependent apoptotic effect, which greatly reduced cancer cell viability. The results proved that the Syzygium cumini phytochemicals are not only intrinsically anticancer-acting but also reducing

and stabilizing agents in nanoparticles synthesis, and thus, improving the therapeutic effect. The researchers concluded that the targeted treatment of cancer based on such bioinspired nanomaterials may be a new approach to the biological potential of plant extracts in combination with nanotechnology. These findings re-enforced the significance of apoptosis-mediated cytotoxicity as a key mechanism of the anticancer activity of Syzygium cumini.

III. MATERIALS AND METHODS

The aim of the current study was to assess the pharmacology of the aqueous seed extract of Syzygium cumini that focuses on its anticancer and anti-inflammatory properties in human carcinoma cell lines. The standard procedures of conducting in vitro experiments were observed in order to have reproducibility and reliability of the findings.

3.1. Research Design

The research was conducted based on experimental in vitro design, using controlled experiments in the laboratory to examine cytotoxic, apoptotic and anti-inflammatory effects of aqueous seed extract on the chosen human cancerous cell lines.

3.2. Sample Details

Experimental models were human carcinoma cell lines (HeLa (cervical carcinoma) and MCF-7 (breast carcinoma). Control of the extract was done using normal fibroblast cells. Syzygium cumini seeds were gathered and identified by a botanist, dried in the shade, ground into a powder and aqueously extracted.

3.3. Instruments and Materials Used

- **Cell culture facilities:** CO_2 incubator, laminar air flow hood, inverted phase-contrast microscope.
- **Reagents and kits:** Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), MTT assay kit, ELISA kits.
- **Analytical instruments:** Spectrophotometer, microplate reader, and fluorescence microscope.

3.4. Procedure and Data Collection Methods

The aqueous seed extract was prepared by hot water maceration and concentrated using a rotary evaporator. Cells were cultured under standard conditions and treated with varying concentrations of the extract for 24–72 hours.

- **Cytotoxicity** was measured using the MTT assay.
- **Apoptosis induction** was assessed through annexin V/PI staining and fluorescence microscopy.
- **Anti-inflammatory activity** was evaluated by quantifying levels of pro-inflammatory cytokines (IL-6, TNF-α) using ELISA after lipopolysaccharide (LPS) stimulation of carcinoma cells.

All experiments were conducted in triplicates to ensure statistical reliability.

3.5. Data Analysis Techniques

Quantitative information was presented in form of mean and standard deviation. One-way ANOVA, then the post hoc test of Tukey, was used as a measure of statistical significance ($p < 0.05$).

IV.RESULT AND DISCUSSION

Syzygium cumini aqueous seed extract was tested on its anticancer and anti-inflammatory properties in the human carcinoma cell lines. Findings are as illustrated below focusing on cytotoxicity, apoptosis and cytokine modulation. Each of the values denotes the average ± SD of the triplicate experiments.

4.1. Cytotoxicity of Extract on Carcinoma Cells

The MTT assay revealed a dose-dependent cytotoxic effect of the extract on HeLa and MCF-7 cells, while normal fibroblast cells exhibited minimal toxicity.

Table 1: Descriptive Statistics of Cell Viability (%) under Different Concentrations of *Syzygium cumini* Aqueous Seed Extract

Cell Line	Concentration (µg/mL)	Mean Viability (%)	Std. Deviation
HeLa	Control	98.2%	1.21
HeLa	50	72.6%	2.31
HeLa	100	49.4%	2.89
HeLa	200	28.1%	2.12
MCF-7	Control	97.8%	1.45
MCF-7	50	75.3%	1.98
MCF-7	100	53.7%	2.56

MCF-7	200	31.5%	2.77
Fibroblast	Control	99.1%	0.98
Fibroblast	200	91.7%	1.87

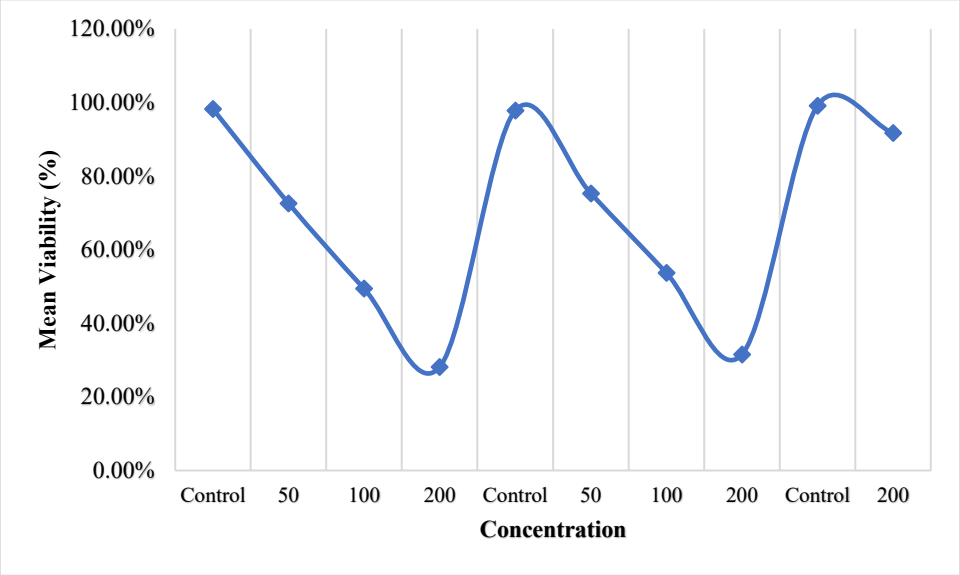

Figure 1: Dose–Response Cytotoxicity Curve

Table 1 shows that aqueous seed extract of Syzygium cumini had a more pronounced and significant dose-dependent reduction of cell viability of both HeLa and MCF-7 carcinoma cells. Viability fell to almost 28 percent in HeLa and 31 percent in MCF-7 cells at 200 µg/mL, but fibroblast cells maintained a viability of more than 90 percent at the highest concentration. This implies that the extract selectively kills cancerous cells and leaves normal cells alive, which makes it an effective anti-cancer compound with less side effects.

4.2. Apoptosis Induction in Carcinoma Cells

Flow cytometry with annexin V/PI staining showed increased apoptosis in a dose-dependent manner, with maximum apoptotic cell death observed at 200 µg/mL.

Table 2: Descriptive Statistics of Apoptotic Index (%) in Treated Cells

Cell Line	Concentration (µg/mL)	Mean Apoptosis (%)	Std. Deviation
HeLa	Control	4.2%	0.56
HeLa	100	28.9%	1.85
HeLa	200	54.6%	2.14
MCF-7	Control	5.1%	0.72
MCF-7	100	32.7%	2.09
MCF-7	200	57.3%	2.45

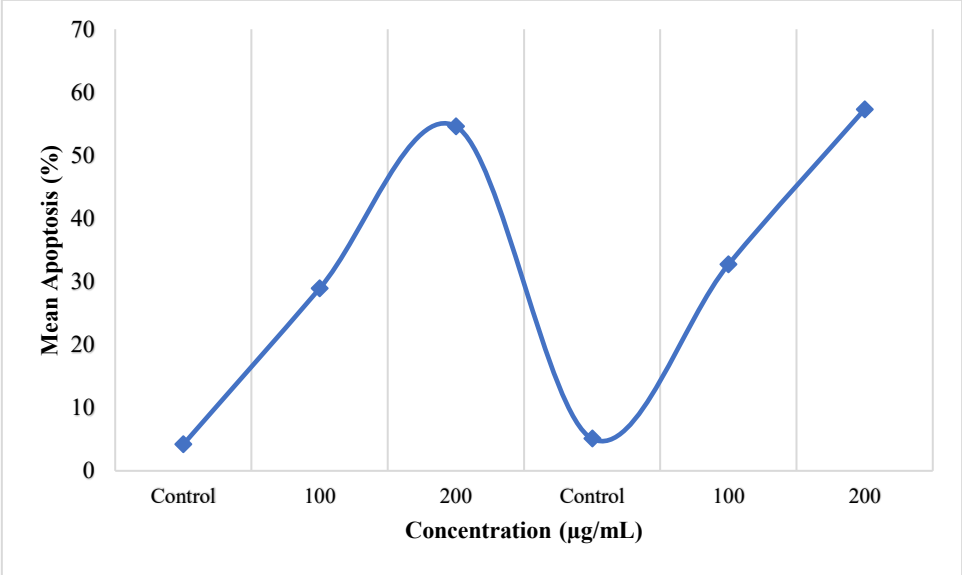

Figure 2: Visual Representation of Mean Apoptosis (%)

Table 2 has indicated the apoptotic index as 1.16, 1.03 and 0.98 respectively, indicating that Syzygium cumini extract caused apoptosis in both HeLa and MCF-7 cells. Control groups had very low levels of baseline apoptosis (approximately 4 to 5 percent), whereas treatment with extract 100 µg/mL in HeLa and MCF-7 cells took the apoptosis levels to approximately 29 percent and 33 percent, respectively. When apoptosis increased to more than 54 percent in HeLa and 57 percent in MCF-7, the apoptotic rates at 200 µg/mL were tremendous. These findings indicate that programmed cell death (apoptosis) was the main mediator of the viability decrease and not nonspecific necrosis.

4.3. Anti-Inflammatory Activity: Cytokine Modulation

ELISA results indicated significant downregulation of IL-6 and TNF-α in extract-treated carcinoma cells compared to LPS-stimulated controls.

Table 3: Cytokine Levels (pg/mL) in LPS-Stimulated Carcinoma Cells

Cytokine	Cell Line	Control (LPS)	Extract 100 µg/mL	Extract 200 µg/mL
IL-6	HeLa	248.3 ± 5.7	142.1 ± 4.2	86.7 ± 3.5
IL-6	MCF-7	231.4 ± 6.2	138.6 ± 3.9	81.2 ± 2.8
TNF-α	HeLa	185.6 ± 4.8	109.2 ± 3.6	64.8 ± 2.3
TNF-α	MCF-7	178.9 ± 5.2	104.7 ± 3.1	59.6 ± 2.1

Table 3 reveals that pro-inflammatory cytokines IL-6 and TNF- a, which are markers of inflammatory disease, were significantly reduced using Syzygium cumini aqueous seed extract in LPS-stimulated carcinoma cells. The level of cytokines in both HeLa and MCF-7 lines reduced in a concentration dependent type and about 6065 percent was inhibited by 200 g/ml. This proves the anti-inflammatory effect of the extract indicating that it may be able to regulate tumor-related inflammation which in most cases leads to cancer development.

4.4. Statistical Analysis

To test the significance of treatment effects, one-way ANOVA followed by Tukey's post hoc test was performed ($p < 0.05$).

Table 4: ANOVA Summary for Cell Viability (HeLa Cells)

Source	Sum of Squares	df	Mean^2	F	Sig.
Between Groups	9821.3	3	3273.8	256.41	.002
Within Groups	102.3	8	12.8		
Total	9923.6	11			

Table 4 shows the summary of one-way ANOVA of HeLa cell viability, in which the F-value (256.41) and the significant level of p (0.001) indicate that the difference between treatment groups is highly significant. This statistical data confirms the hypothesis that different cell concentrations of the extract exerted a real and quantifiable effect on cell viability eliminating the possibility of random variability being the cause of observed effects.

Table 5: Post Hoc Test (Tukey HSD) for HeLa Cell Viability

(I) Concentration	(J) Concentration	Mean Difference (I-J)	Sig.
Control	50 µg/mL	25.6*	.000
Control	100 µg/mL	48.8*	.000
Control	200 µg/mL	70.1*	.000
50 µg/mL	100 µg/mL	23.2*	.001
100 µg/mL	200 µg/mL	21.3*	.002

Table 5 presents a post hoc analysis that indicates that all the concentrations of the extract yielded statistically significant decrease in the viability of HeLa cells compared to the control. Moreover, changes (50 vs. 100 µg/mL and 100 vs. 200 µg/mL) between intermediate concentrations were also noteworthy, which testifies to a dose dependence pattern. This supports the fact that Syzygium cumini extract is gradually decreasing cancer cell survival with increase of dose.

4.5. Discussion

The current paper has examined the pharmacological prospects of Syzygium cuminii aqueous seed extract with specific reference to its anticancer and anti-inflammatory actions on human carcinoma cell lines. These findings showed clearly that the extract was selectively cytotoxic to HeLa and MCF-7 cells, caused dose-dependent apoptosis, and reduced pro-inflammatory cytokines of IL-6 and TNF-alpha significantly. Collectively, these results indicate that Syzygium cumini has some potential bioactivities that can be used to manage cancer by not only affecting the survival of the tumor cells but also the linked inflammatory response.

Interpretation of Results

The extract showed clear anticancer and anti-inflammatory activities.

- **Cytotoxicity:** A marked reduction in carcinoma cell viability was observed with increasing concentrations of the extract, while normal fibroblast cells remained largely unaffected. This selectivity indicates that the extract has potential as a safer therapeutic option compared to conventional cytotoxic agents.
- **Apoptosis Induction:** The significant increase in apoptotic index at higher concentrations confirmed that the decrease in cell viability was mediated through programmed cell death rather than nonspecific necrosis. This mechanism is highly desirable for anticancer agents.
- **Cytokine Modulation:** A substantial anti-inflammatory action of the extract was demonstrated by the dose-dependent decrease of IL-6 and TNF-α in carcinoma cells treated with LPS. This dual effect enhances Syzygium cumini's therapeutic value, as chronic inflammation is a key factor in cancer progression.
- **Statistical Validation:** ANOVA and Tukey's post hoc analysis confirmed that the observed effects were highly significant, reinforcing the robustness of the experimental outcomes.

Implications of Findings

The outcomes provide both therapeutic and scientific significance.

- The selective cytotoxicity against carcinoma cells suggests potential use of *Syzygium cumini* seed extract as a natural adjunct or alternative in cancer therapy.
- The observed apoptosis indicates that bioactive compounds in the extract may target molecular pathways regulating programmed cell death, warranting further mechanistic exploration.
- The anti-inflammatory activity underscores its value in modulating tumor microenvironments, thereby potentially reducing cancer aggressiveness and resistance.
- These results support the ethnomedicinal use of *Syzygium cumini* and provide a scientific basis for its pharmacological applications in oncology and immunomodulation.

Limitations of the Study

Certain constraints must be acknowledged.

- The study was limited to in vitro models; results may differ in complex in vivo systems.
- Only two carcinoma cell lines were tested, which may not represent the full spectrum of cancer types.
- The specific bioactive compounds responsible for the observed effects were not isolated or identified.
- The molecular signaling pathways involved in apoptosis and cytokine suppression were not elucidated.
- Dose–response studies were restricted to short-term exposures (24–72 hours), limiting long-term outcome predictions.

Suggestions for Future Research

Further investigations can strengthen and expand these findings.

- Conduct in vivo studies to validate anticancer and anti-inflammatory effects in animal models.

- Perform bioassay-guided fractionation to identify, isolate, and characterize the active phytochemicals responsible for the observed effects.
- Explore molecular mechanisms, particularly apoptotic signaling cascades (e.g., caspase activation, mitochondrial pathways) and inflammatory pathways (e.g., NF-κB inhibition).
- Extend research to other cancer cell lines to assess the broader applicability of the extract.
- Evaluate the potential synergistic effects of *Syzygium cumini* extract when combined with standard chemotherapeutic agents.

V.CONCLUSION

The current study shows that the aqueous seed extract of Syzygium cumini has strong pharmacological potential against human carcinoma cell lines. The extract exhibited dose-dependent cytotoxicity, sparing normal fibroblasts while specifically decreasing viability in HeLa and MCF-7 cells. It demonstrated both anticancer and anti-inflammatory mechanisms by successfully inducing apoptosis and dramatically downregulating the pro-inflammatory cytokines TNF-α and IL-6. These results support Syzygium cumini's traditional medical use and raise the possibility that its bioactive components could be a promising natural cancer treatment.

In order to verify the extract's effectiveness and safety in animal models, more investigation should concentrate on in vivo studies. In addition to a thorough investigation of molecular mechanisms like inflammatory signaling and apoptosis pathways, active phytochemicals with anticancer and anti-inflammatory properties must be isolated and characterized. Its potential as an adjuvant therapy for the treatment of cancer may also be revealed by examining synergistic effects with conventional chemotherapeutic agents.

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