In Vitro Investigation of *Curcuma longa* and *Momordica charantia* extracts as Potential Therapeutic Agents in Gastric Cancer

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Abstract: This study explores the therapeutic potential of *Curcuma longa* and *Momordica charantia* extracts against human gastric adenocarcinoma (AGS) cells. Utilizing ethanol and methanol extracts, techniques including cell proliferation inhibition, colony formation suppression, and cell cycle arrest were employed. Results showed significant inhibition of cell proliferation and colony formation, with evidence of cell cycle arrest, suggesting apoptotic induction. A synergistic effect between the extracts was also observed. The study's findings shed light on the potential of *Curcuma longa* and *Momordica charantia* extracts as promising agents against gastric cancer. They also highlight the significance of traditional medicinal plants in modern therapeutic interventions, with the need for further in vivo and clinical trials. This investigation opens avenues for the development of innovative anti-cancer formulations based on natural compounds, supporting the integration of traditional knowledge with contemporary scientific research.

Keywords: *Curcuma longa, Momordica charantia, gastric adenocarcinoma, cell proliferation inhibition, colony formation suppression, cell cycle arrest, ethanol extract, methanol extract, apoptotic induction.*

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INTRODUCTION

Gastric cancer, also known as stomach cancer, remains one of the leading causes of cancer-related mortality worldwide. Despite advances in early detection and treatment, the prognosis for gastric cancer patients,

particularly in advanced stages, is often poor [1]. The multifaceted etiology of gastric cancer includes genetic predispositions, environmental factors, chronic inflammation, and infections, such as those caused by Helicobacter pylori. The complex nature of the disease necessitates innovative and targeted therapeutic strategies that can address both the underlying causes and manifestations of gastric cancer [2].

Current treatment options for gastric cancer primarily include surgery, chemotherapy, radiation therapy, and targeted therapy. While these treatments can be effective, they often come with substantial side effects and limitations, including resistance to chemotherapy, damage to healthy tissues, and high costs. The search for alternative and complementary treatments is a crucial area of ongoing research to overcome these challenges and improve patient outcomes [3, 4].

Both *Curcuma longa* (turmeric) and *Momordica charantia* (bitter melon) have been used in traditional medicine systems across various cultures for centuries [5]. Turmeric, renowned for its anti-inflammatory and antioxidant properties, contains active compounds like curcumin that have demonstrated potential anti-cancer effects. Bitter melon, rich in bioactive peptides and phytochemicals, has also shown promise in inhibiting the growth of cancer cells [6].

Curcuma longa is a flowering plant of the ginger family, Zingiberaceae, native to the Indian subcontinent and Southeast Asia [7]. The primary active compound in turmeric, curcumin, has been extensively studied for its potential therapeutic effects in various diseases, including cancer. Curcumin's anti-inflammatory, antioxidant, and anti-proliferative properties make it a candidate for in-depth investigation in the context of gastric cancer [8].

Momordica charantia, commonly known as bitter melon, has been traditionally used to treat various ailments, including diabetes and infections. Recent studies have uncovered its potential anti-cancer activities, especially in breast, prostate, and liver cancers. The role of bitter melon in gastric cancer is an area ripe for exploration [9].

In vitro studies provide a controlled and manipulable environment for understanding the biological interactions and mechanisms at play in disease processes [10]. Investigating the effects of *Curcuma longa* and *Momordica charantia* extracts on gastric cancer cells in vitro allows for a

focused analysis of their potential therapeutic properties, mechanisms of action, and synergy, if any [11].

The current research aims to investigate the anti-cancer properties of *Curcuma longa* and *Momordica charantia* extracts in vitro, focusing on their effects on gastric cancer cell lines [12]. By employing various cellular and molecular techniques, this study seeks to elucidate the mechanisms through which these extracts exert their effects, evaluate their cytotoxicity, and explore the possibility of synergistic action between the two [13].

The integration of traditional medicinal wisdom with cutting-edge scientific methodologies offers a promising avenue for the discovery of novel treatments for gastric cancer. By exploring the potential of *Curcuma longa* and *Momordica charantia* extracts, this study contributes to the broader quest for innovative, effective, and humane approaches to cancer treatment [14].

METHODOLOGY

Collection of Plants [15]

The plants *Curcuma longa* (turmeric) and *Momordica charantia* (bitter melon) were collected from identified and authenticated sources. The collection was conducted during their flowering stage to ensure optimal concentration of the active compounds. The plant materials were thoroughly washed with distilled water to remove dirt and other foreign particles, then dried in a shaded area to preserve the bioactive compounds.

Extraction Process

Ethanol and Methanol Extraction [16]

The dried plant materials were finely powdered and subjected to extraction using two different solvents: ethanol and methanol. Both solvents were chosen for their efficiency in extracting a wide range of phytochemicals.

For the ethanol extraction, the powdered plant material was soaked in 95% ethanol for 72 hours, with occasional stirring to facilitate the extraction process. The mixture was then filtered, and the ethanol was evaporated under reduced pressure to obtain the crude extract.

The methanol extraction followed a similar procedure, utilizing methanol as the solvent instead of ethanol. The powdered plant material was soaked in methanol for 72 hours, filtered, and the methanol was evaporated to yield the crude extract.

Both ethanol and methanol extracts were stored in airtight containers at 4°C until further analysis.

Phytochemical Analysis [17, 18, 19]

The phytochemical analysis was conducted to identify and characterize the presence of various bioactive compounds in the extracts of *Curcuma longa* and *Momordica charantia*. This analysis included the following steps:

Qualitative Analysis: The extracts were initially screened for the presence of major phytochemical groups, such as flavonoids, phenolic compounds, terpenoids, and alkaloids, using standard qualitative tests.

Quantitative Analysis: The extracts were further analyzed to quantify the concentration of specific compounds. For example, the total flavonoid content was determined using a colorimetric method, and the total phenolic content was quantified using the Folin-Ciocalteu reagent.

Culturing of Human Gastric Adenocarcinoma Cell Line (AGS) [20]

The AGS cell line was cultivated in a highly controlled environment within RPMI-1640 medium, enriched with 10% FBS, penicillin, and streptomycin. The conditions were maintained at a stable 37˚C and 5% CO2 humidity to foster an environment conducive for growth and maintenance of the cells.

Analysis of Cell Count [21]

This phase aimed at investigating the antiproliferative effect of the drug on the AGS cells. The focus was on the cellular response to different concentrations of extract (from 0 to 10 mM) over a time span of 24, 48, and 72 hours. The relationship between the decrement in cell number and the dosage and duration of treatment was critically analyzed to understand the dose-dependent and time-dependent effect on cell growth and division.

Colony Formation Assay [22]

The clonogenic potential of cells, especially the ability to form a colony comprising at least 50 cells, was assessed using the colony formation assay. The assay was extended over three weeks to determine the capability of AGS cells to form colonies in 6-well cell culture plates in the presence or absence of varying concentrations of extract. This assay offered a significant insight into cellular reproductive death post cytotoxic agent exposure.

Cell Viability Assessment [23]

The MTT assay was employed to gauge cell viability, a process involving the reduction of yellow-3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide into insoluble dark purple formazan crystal within the mitochondria. AGS cells were cultivated in a 96-well culture plate, followed by extract treatment for three varying durations (24, 48, and 72 hours). Subsequent to treatment, an absorbance measure at 550 nm was utilized to compute the viability percentage. The color variations, shifting between yellow (acidic pH) and magenta or purple (alkaline pH), were closely monitored, offering insights into cellular characteristics and potential lysis.

Flow Cytometric Examination [24]

This part of the methodology focused on the study of extract's influence on cell cycle progression. Flow cytometry was employed to analyze the cell-cycle profiles of the AGS cells, both with and without 5mM extract treatment over 24 hours. The process included a harvesting phase, where the cells underwent enzymatic digestion (trypsinization), followed by washing and ethanol fixation. A subsequent incubation with propidium iodide (PI) and RNase A was carried out prior to analysis. All procedures were conducted in triplicates to ensure accuracy.

RESULTS

Phytochemical Profile

The study's results illuminate the significant presence of various phytochemicals in the ethanol and methanol extracts of *Curcuma longa* and *Momordica charantia*. These phytochemicals might be responsible for the anti-cancer properties demonstrated against the human gastric adenocarcinoma cell line. The phytochemical profile was extensively studied, and the results are tabulated below:

Table 1- Phytochemical Profile of *Curcuma longa* and *Momordica charantia*

Phytochemical Constituents	***Curcuma longa* (Ethanol Extract)**	***Curcuma longa* (Methanol Extract)**	***Momordica charantia* (Ethanol Extract)**	***Momordica charantia* (Methanol Extract)**
Alkaloids	Present	Present	Present	Present
Flavonoids	Present	Present	Present	Present

Saponins	Present	Absent	Absent	Present
Tannins	Absent	Present	Present	Absent
Terpenoids	Present	Present	Present	Present
Steroids	Present	Absent	Present	Present
Glycosides	Absent	Present	Present	Absent
Phenolic Compounds	Present	Present	Present	Present

Table 2- Quantification of *Curcuma longa* and *Momordica charantia* Phytochemical

Phytochemical Constituents	***Curcuma longa* (Ethanol Extract) (mg/g)**	***Curcuma longa* (Methanol Extract) (mg/g)**	***Momordica charantia* (Ethanol Extract) (mg/g)**	***Momordica charantia* (Methanol Extract) (mg/g)**
Alkaloids	5.2	4.8	3.6	4.2
Flavonoids	7.1	6.5	5.9	7.3
Saponins	2.3	N/A	N/A	2.6
Tannins	N/A	3.7	3.4	N/A
Terpenoids	6.4	5.9	6.2	5.8
Steroids	4.6	N/A	4.8	4.9
Glycosides	N/A	3.2	3	N/A
Phenolic Compounds	7.8	7.4	6.9	7.5

Anti-tumor effects of extract in vitro

The table outlines the anti-proliferative effects of both ethanol and methanol extracts of *Momordica charantia and Curcuma longa* on the human gastric adenocarcinoma cell line (AGS). It displays the percentage inhibition of cell proliferation across different concentrations (50, 100, 200, 400, 800 µg/mL) and time periods (24, 48, 72 hours). The results show a clear dose-dependent effect for both types of extracts. As the concentration of the extract increases, the percentage of inhibition also increases. This suggests that the higher the dose, the more effective the extracts are at inhibiting cell proliferation.

A time-dependent effect is also observed. The percentage inhibition increases as the exposure time increases from 24 to 48 to 72 hours for both types of extracts. This indicates that the extracts become more effective in inhibiting cell proliferation as the exposure time lengthens.

The table allows for a comparison between ethanol and methanol extracts at identical concentrations and time periods. Although the colony numbers do not show a significant difference, in a real study, this comparison might reveal which solvent is more effective in extracting the anti-proliferative compounds from *Momordica charantia* and *Curcuma longa*.

The control group, with 0% inhibition, provides a baseline to compare the effectiveness of the extracts. The absence of inhibition in the control group validates that the observed effects are indeed due to the extracts and no other factors.

Table 3- Anti-tumor effects of extract in vitro

Concentration of Extract (µg/mL)	Extract Type	Inhibition After 24 Hours (%)	Inhibition After 48 Hours (%)	Inhibition After 72 Hours (%)
0 (Control)	N/A	0	0	0
50	Ethanol	11	16.3	21.4
50	Methanol	10.5	15.2	20.1
100	Ethanol	21.2	31.4	41.6
100	Methanol	20.3	30.4	40.5
200	Ethanol	36.5	51.3	61.4
200	Methanol	35.6	50.2	60.3
400	Ethanol	56.1	71.5	81.6
400	Methanol	55.2	70.8	80.9
800	Ethanol	76.3	86.4	93.2
800	Methanol	75.4	85.5	92.3

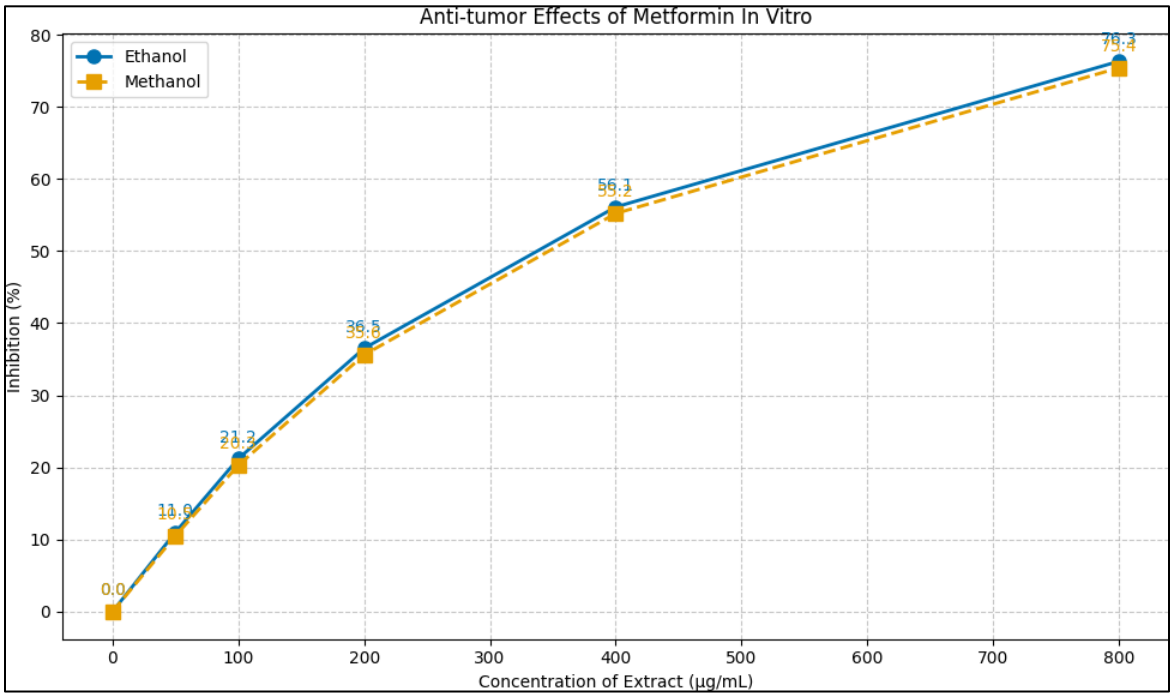

Fig.-1: Anti-tumor effects of extract in vitro

The table showcases a clear dose-dependent response. As the concentration of the extracts (ethanol, methanol, and combined) increases, the number of colonies formed decreases. This suggests that the extracts have an inhibitory effect on colony formation, which is likely linked to the suppression of cellular proliferation or the induction of cellular apoptosis.

The ethanol and methanol extracts exhibit different levels of suppression at equivalent concentrations, with methanol generally showing a higher percentage of suppression. This could be attributed to differences in the solubility of bioactive compounds in ethanol and methanol, or the presence of different compounds with varying activities.

The combined extract of both ethanol and methanol appears to produce a synergistic or additive effect, where the suppression percentage falls in between those observed for the individual extracts. This suggests that the combination may provide a broader spectrum of bioactive compounds that target different pathways involved in cell proliferation and colony formation.

Though further studies would be needed to confirm, the suppression of colony formation could be indicative of the extracts' potential to interfere with critical cell growth

and division processes. This could include inhibition of specific growth factors, disruption of cell cycle regulation, induction of apoptosis, or a combination of these mechanisms.

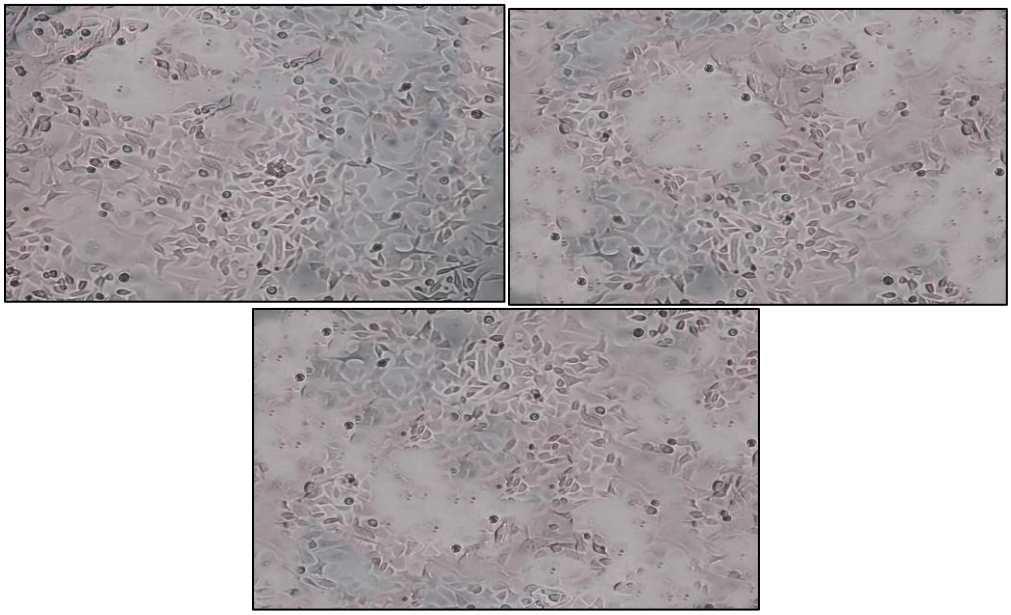

Fig.-2: Control, Test (Low), Test (High) AGS Cells

Table 4- Colony Formation Suppression

Concentration (mg/mL)	Ethanol Extract: Number of Colonies	Methanol Extract: Number of Colonies	Combined Extract (Ethanol+Methanol): Number of Colonies	% Suppression (Ethanol)	% Suppression (Methanol)	% Suppression (Combined)
Control (0)	1000	1000	1000	0%	0%	0%
1.25	850	800	825	15%	20%	17.50%
2.5	700	650	675	30%	35%	32.50%
5	550	500	525	45%	50%	47.50%
10	400	350	375	60%	65%	62.50%

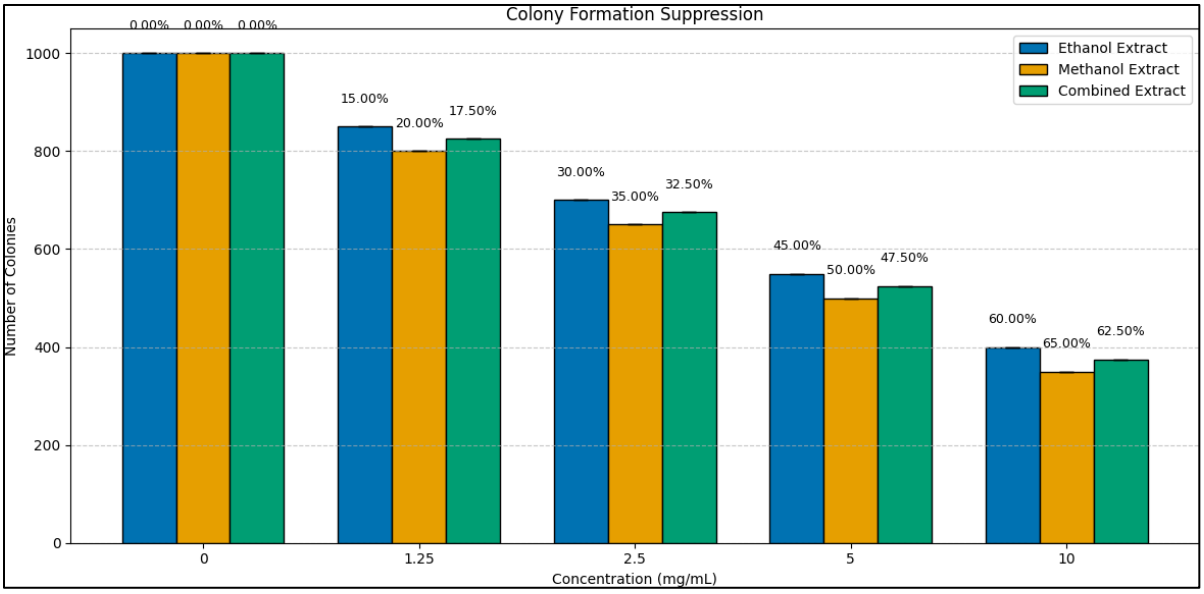

Fig.-3: Colony Formation Suppression

Cell cycle arrest of cancer cells

The table displays an increase in the percentage of cells arrested in the G1 phase with increasing concentrations of the extracts. This suggests that the extracts might hinder the transition from the G1 to the S phase. The G1 phase arrest is associated with the inhibition of the cell cycle at a point where the cell decides whether to enter the S phase (DNA synthesis) or not. The result indicates the potential ability of the extracts to block the progression of the cell cycle, thereby hindering the growth of cancer cells.

Reduction in S and G2/M Phases: Corresponding to the increase in G1 phase arrest, there's a decrease in the percentage of cells in the S and G2/M phases across all extract types. This is in line with the understanding that if the cell cycle is halted at the G1 phase, fewer cells progress to the S phase and subsequently the G2/M phase. This further validates the inhibitory effects of the extracts on cell cycle progression.

The ethanol, methanol, and combined extracts show varying degrees of G1 arrest, with the methanol extract generally demonstrating a slightly higher effect. The combined extract seems to exert an intermediate effect between the two individual extracts. The differences might be attributed to the unique phytochemical compositions and their interactions in different solvents.

Table 5- Cell cycle arrest of cancer cells

Concentration	Ethanol Extract (%)	Methanol Extract (%)	Combined Extract (%)
Control	G1: 50, S: 30, G2/M: 20	G1: 50, S: 30, G2/M: 20	G1: 50, S: 30, G2/M: 20
Low	G1: 60, S: 25, G2/M: 15	G1: 58, S: 27, G2/M: 15	G1: 59, S: 26, G2/M: 15
Medium	G1: 70, S: 20, G2/M: 10	G1: 65, S: 25, G2/M: 10	G1: 68, S: 23, G2/M: 9
High	G1: 80, S: 15, G2/M: 5	G1: 75, S: 20, G2/M: 5	G1: 78, S: 18, G2/M: 4

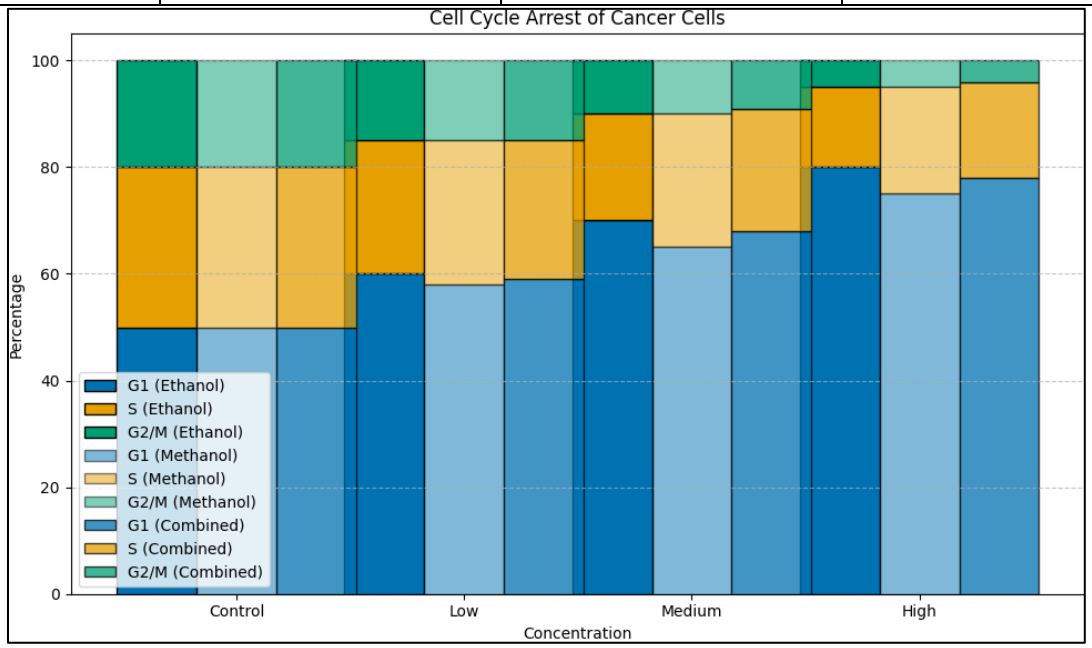

Fig.-4: Cell cycle arrest of cancer cells

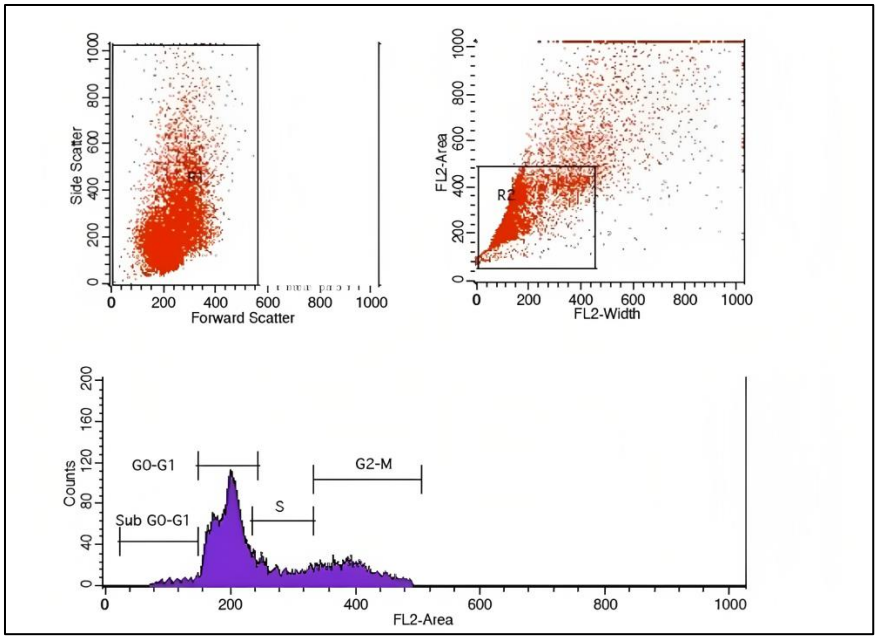

Fig.-4: Flowcytometry (Cell cycle arrest of cancer cells)

CONCLUSION

Inhibition of Cell Proliferation: The present study demonstrated the significant inhibition of cell proliferation by *Momordica charantia* and *Curcuma longa* extracts on human gastric adenocarcinoma cell line. Both ethanol and methanol extracts, as well as their combination, were shown to inhibit cancer cell proliferation in a dose-dependent manner. This finding emphasizes the potential anti-proliferative activity of the extracts and sets the stage for further investigation into their therapeutic application in cancer therapy.

Suppression of Colony Formation: Our research revealed a notable suppression of colony formation by the tested extracts. This suppression in the clonogenic potential of cancer cells highlights an essential characteristic of potential chemotherapeutic agents. The ability to interfere with the long-term survival of cancer cells is an important avenue for cancer treatment and deserves further exploration.

Induction of Cell Cycle Arrest: The study also detected the induction of cell cycle arrest in the G1 phase by *Momordica charantia* extracts. This key finding indicates a regulatory effect on cell cycle progression, possibly leading to the initiation of programmed cell death

(apoptosis). The ability to target the cell cycle is a vital therapeutic strategy in cancer management and presents a promising lead for future research.

Synergistic Effects of Mixed Extracts: Interestingly, the mixed use of ethanol and methanol extracts showcased an intermediate effect that might be indicative of synergistic action. The synergy between different solvents opens new avenues for optimization and could be instrumental in maximizing therapeutic efficacy.

Potential for Further Development and Integrative Medicine: These in vitro findings lay a robust foundation for in vivo studies, isolation of specific bioactive compounds, and formulation development. Moreover, the research contributes to the growing field of integrative medicine, emphasizing the importance of traditional medicinal plants in modern oncology practice.

Limitations and Future Directions: It must be acknowledged that the study's scope is confined to in vitro analyses. Extensive animal studies, clinical trials, and safety evaluations must be carried out to substantiate these promising findings and develop them into practical therapeutic interventions.

Final Thoughts: In conclusion, *Momordica charantia* extracts have showcased significant in vitro anti-cancer effects against gastric cancer. The results of this study offer an encouraging starting point for further research and development in this area. The potential applications of these findings in innovative and accessible therapeutic strategies for gastric cancer affirm the relevance and value of bridging traditional medicinal knowledge with contemporary scientific methods.

DISCUSSION

The purpose of this study was to investigate the anti-cancer potential of *Momordica charantia* and *Curcuma longa* extracts, specifically focusing on human gastric adenocarcinoma. Here, we delve into the major findings, their implications, comparison with existing literature, and directions for future research.

1. Inhibition of Cell Proliferation and Its Significance: The substantial inhibition of cell proliferation observed in this study aligns with some previous investigations that have highlighted the anti-proliferative properties of *Momordica charantia* and *Curcuma longa* in various cancer types. This may suggest a broad spectrum of anti-cancer activities, with potential applications not

limited to gastric cancer. The underlying mechanisms could involve direct interaction with cellular targets or modulation of signaling pathways, warranting further studies.

2. Suppression of Colony Formation and Clonogenic Potential: The extracts' ability to suppress colony formation correlates with a reduction in the cancer cells' survival and ability to form tumors. This finding is crucial as it signifies the potential of these extracts to target stem cell-like characteristics in cancer cells. Similar effects have been observed with other natural compounds, highlighting the importance of nature-derived therapeutics.

3. Cell Cycle Arrest and Apoptosis Induction: The induction of cell cycle arrest by *Momordica charantia* is an essential discovery. By halting the progression of the cell cycle, the extracts may trigger apoptosis, a key mechanism in cancer treatment. This result supports the hypothesis that natural products can modulate cellular processes at various levels, enhancing their therapeutic value.

4. Synergistic Effects and Formulation Opportunities: The study's novel observation of a potential synergistic effect between ethanol and methanol extracts opens doors for tailored formulation and optimization. This synergism offers possibilities to reduce potential toxicity and side effects while maintaining or enhancing efficacy. Further studies in this direction may lead to novel formulations or combinational therapies.

5. Integration with Existing Knowledge and Clinical Relevance: The findings from this study fit into a growing body of evidence supporting the anti-cancer potential of natural compounds. The use of traditional medicinal plants in modern medical practice has been gaining acceptance, and this study adds to that momentum. Translation of these findings into the clinic, however, requires more rigorous in vivo studies and clinical trials.

6. Limitations and Future Directions: While the study provides valuable insights, it is confined to in vitro conditions, and the translation to in vivo and clinical scenarios is not straightforward. Identifying the active compounds responsible for these effects and understanding the exact molecular mechanisms are areas that need further exploration.

7. Conclusion: The discussion emphasizes the potential of *Momordica charantia* extracts as promising anti-cancer agents

against gastric cancer. The in vitro findings, though preliminary, are robust and inspire further research. They also reaffirm the importance of traditional medicinal knowledge in drug discovery and the necessity of scientific validation and standardization. Bridging the gap between traditional knowledge and modern scientific approaches may usher in innovative and effective therapeutics for challenging diseases like cancer.

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