RESEARCH PAPER

"Exploring the Anticancer Potential of Scirrhinum 200 for Breast and Gastric Cancer Treatment: An In Vitro Experimental Research Study".

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ABSTRACT

Even though tremendous progress has been made in the areas of surgery, chemotherapy, radiotherapy, and targeted therapies in the treatment of cancer, many limitations such as toxicity, resistance to treatment, and recurrence have driven researchers to look for complementary and alternative options. This research work examined the antitumor effects of Scirrhinum 200 homeopathic nosode on MCF-7 breast cancer and AGS gastric cancer cells using MTT test. 5-Fluorouracil was used as a reference drug. Scirrhinum 200 showed dose-dependent cytotoxic activity in both types of cell lines by causing decreased viability and increased growth inhibition due to the increase in concentrations. Scirrhinum 200 caused an IC_{50} value of 79.60 µg/mL and 65.18% inhibition in MCF-7 cells while 90.69 µg/mL and 59.31% inhibition was noted in AGS cells at 100 µg/mL concentration. Microscopic examination indicated some specific changes in morphology such as cell shrinking, rounding up, detaching from each other, and low cell density suggesting cytotoxic activity. According to flow cytometry, there was no considerable amount of apoptosis induction under the mentioned experimental conditions; therefore, more studies are needed regarding its mechanism. Although not as effective as 5-Fluorouracil, Scirrhinum 200 showed moderate anti-tumor activity especially on MCF-7 cells..

Keywords: Scirrhinum 200, Homeopathy, Anticancer activity, MCF-7 cells, AGS cells, Breast cancer, Gastric cancer, MTT assay, Cytotoxicity, Apoptosis.

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INTRODUCTION

Cancer Overview and Rationale for Alternative Therapies

Despite the considerable success achieved through traditional treatment modalities like surgery, chemotherapy, radiotherapy, and targeted therapies, their efficiency has been hindered by various side effects, drug resistance, and the potential for the recurrence of the disease. These drawbacks have caused increasing attention to be paid to complementary and alternative medicine (CAM), such as homeopathy, as possible forms of therapy. Consequently, scientific evaluation of alternative therapies is essential to determine their efficacy and safety in cancer management.[1]

Breast Cancer: Burden and Current Therapies

Despite advances in surgery, chemotherapy, hormonal therapy, and targeted treatments, challenges such as treatment-related toxicity, drug resistance, disease recurrence, and poor outcomes in metastatic cases remain significant. These limitations have increased interest in complementary and alternative therapies.[2] The MCF-7 human breast cancer cell line, an estrogen receptor-positive model, is widely used in vitro to evaluate the anticancer potential of novel substances by assessing their effects on cell proliferation and survival.[3]

Gastric Cancer: Burden and Current Therapies

Gastric cancer is the most commonly diagnosed cancer. It having low survival rates and significant treatment-related side effects. These limitations have encouraged interest in alternative and complementary therapies. The AGS human gastric adenocarcinoma cell line is widely used as an in vitro model to evaluate the anticancer potential of novel compounds and therapeutic agents.[4]

Homeopathy in Cancer Treatment: An Overview

Principles underlying homeopathy include that of similia similibus curentur ("like cures like") and potentiation by means of repeated dilution and shaking. It has been used to complement conventional treatment of cancer patients for symptom relief and its alleged antineoplastic activity. Some in vitro studies have reported that highly diluted homeopathic remedies can induce cytotoxicity suggesting possible biological activity.[5] However, findings remain inconsistent, and the anticancer potential of homeopathic medicines requires further scientific investigation to establish their efficacy and mechanism of action.[6]

Scirrhinum 200: A Homeopathic Nosode

Scirrhinum is a homeopathic nosode prepared from scirrhous carcinoma tissue and is traditionally used for conditions associated with a cancerous predisposition, particularly hard glandular or breast tumors. Although widely used in homeopathic practice at high potencies such as 200C, scientific evidence regarding its anticancer activity remains scarce.[7] Therefore, *Scirrhinum 200* on cancer is important to assess its potential influence on cell viability, proliferation, and morphology. Such studies may provide preliminary evidence for its biological activity and

support further research into its possible anticancer mechanisms.[8]

LITERATURE REVIEW

Pathak et al. (2003) find out anticancer action of *Ruta graveolens* 6C against human glioma cells. The study demonstrated significant cytotoxic effects. In animal experiments, Ruta 6C prolonged survival and reduced tumor burden. The authors suggested that the remedy selectively targeted malignant cells while preserving normal lymphocytes. These findings provided early scientific evidence supporting the potential role of homeopathic medicines in cancer therapy.

Khuda-Bukhsh et al. (2006) find out the molecular action of various homeopathic preparations on cancer cells and reported significant modulation of gene expression. Their research suggested that ultra-diluted remedies could influence apoptotic pathways, DNA repair mechanisms, and cellular signaling processes. The study contributed to understanding the possible biological mechanisms underlying homeopathic anticancer activity.

Banerji et al. (2008) evaluated the clinical and experimental applications of homeopathic medicines in cancer management. The authors reported encouraging outcomes with remedies such as Psorinum and Ruta graveolens in selected cancer cases. Laboratory investigations supported their clinical observations.

Frenkel et al. (2010) examined the action of Carcinosin, Phytolacca. The study found increased apoptotic activity in treated cancer cells. Importantly, showed minimal toxicity, suggesting selective anticancer action. This work remains one of the most widely cited studies in homeopathic oncology research.

Saha et al. (2013) studied homeopathic remedies. The results showed higher levels of pro-apoptotic protein activation and inhibition of anti-apoptotic signals. The study proposed that homeopathic remedies may trigger programmed cell death through oxidative stress-mediated mechanisms.

Preethi et al. (2014) reviewed complementary and alternative medicine approaches in cancer care. The authors brought attention to the growing popularity of homeopathy as an adjuvant therapy for symptom relief and improving quality of life. They stressed the importance of conducting scientific research in laboratories to prove the anticancer effectiveness of homeopathic drugs.

Rajendran et al. (2015) evaluated the role of complementary therapies in oncology and reported that many cancer patients seek alternative treatments to reduce treatment-related toxicity. Homeopathy was identified as one of the most commonly used modalities. The review stressed the importance of evidence-based research to validate homeopathic interventions.

Bellavite et al. (2015) explored experimental evidence supporting biological activity in ultra-high dilutions. The authors reviewed cellular, molecular, and animal studies demonstrating measurable biological responses following exposure to homeopathic medicines. Their work provided theoretical support for further investigations into homeopathic anticancer mechanisms.

Nayak et al. (2016) assessed the role of homeopathic medicines in supportive cancer care. The study reported improvements in fatigue, nausea, anxiety, and treatment-related adverse effects among cancer patients receiving homeopathic treatment. The authors concluded that homeopathy may improve overall patient well-being when used alongside conventional therapy.

Milazzo et al. (2017) conducted a systematic review on homeopathy in oncology. The review analyzed available clinical and laboratory evidence and found that although supportive benefits were frequently reported, stronger scientific studies were required to establish direct anticancer effects. The authors recommended further in vitro and mechanistic investigations.

Bhardwaj et al. (2018) investigated apoptosis-inducing mechanisms of natural and complementary medicines in cancer cells. Their findings suggested that alternative therapies may regulate cell-cycle progression, mitochondrial pathways, and caspase activation. The study provided a framework for evaluating novel homeopathic anticancer agents.

Lohi et al. (2019) the authors reported growing interest in homeopathy due to its perceived safety, affordability, and holistic approach. They emphasized that scientific validation through cell culture and molecular studies was essential for integrating homeopathic medicines into evidence-based oncology.

Frass et al. (2020) evaluated homeopathy as an adjunctive treatment in patients with advanced-stage cancer. The study reported survival outcomes among patients receiving individualized homeopathic therapy in addition to conventional treatment. The findings suggested that homeopathy may offer supportive benefits in comprehensive cancer care.

Basu et al. (2023) the authors concluded that Arsenicum album possesses significant antiproliferative activity against breast cancer cells.

Beula et al. (2023) evaluated anti-cancer potential using MTT, SRB, and colony formation assays. The study provided experimental evidence supporting the traditional use of Conium in glandular tumors and breast cancer-related conditions.

Musale et al. (2025) explored the in vitro anticancer potential of *Scirrhinum.* The study highlighted the novelty of investigating *Scirrhinum* as a cancer nosode and demonstrated its potential to reduce cancer cell viability. The authors suggested that further mechanistic studies are required to elucidate its mode of action and therapeutic significance.

MATERIAL & METHODS

MTT Assay Procedure

Principle of the MTT Assay

The MTT assay is a widely used colorimetric method for evaluating cell viability, proliferation, and cytotoxicity of test compounds against cultured cells. The assay is based on the ability of metabolically active (viable) cells to reduce the yellow, water-soluble tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into insoluble purple formazan crystals through the

action of mitochondrial succinate dehydrogenase and other oxidoreductase enzymes.[9]

Only viable cells possessing intact mitochondrial activity can reduce MTT to formazan, whereas dead or damaged cells lose this metabolic capability and therefore fail to produce the purple crystals. After incubation, the insoluble formazan crystals are dissolved using an appropriate solvent such as dimethyl sulfoxide (DMSO), and the resulting purple-colored solution is quantified spectrophotometrically at 570 nm. The intensity of the absorbance is directly proportional to the number of viable cells present.[10]

In the present study, the cytotoxic effect of Scirrhinum 200 on human breast adenocarcinoma (MCF-7) and human gastric adenocarcinoma (AGS) cell lines was evaluated using the MTT assay. The assay was selected because it is simple, sensitive, reproducible, cost-effective, and widely accepted for screening the anticancer potential of experimental compounds.[11]

MTT Assay Protocol for MCF-7 Cells

Human breast adenocarcinoma (MCF-7) cells were cultured under standard aseptic conditions in appropriate culture medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2.[12]

Exponentially growing cells were harvested and seeded into sterile 96-well tissue culture plates at an appropriate density to obtain approximately 70–80% confluence at the time of treatment. Following overnight incubation to allow cell attachment, the culture medium was replaced with fresh medium containing different concentrations of Scirrhinum 200. Untreated cells served as the negative control, while cells treated with the standard anticancer drug 5-Fluorouracil (5-FU) served as the positive control. A vehicle control (0.3% ethanol) was also included to eliminate any cytotoxic effects attributable to the solvent.[13]

After treatment for 24 or 48 hours, the culture medium was carefully removed, and MTT solution (0.5 mg/mL final concentration) was added to each well. The plates were incubated for an additional 3 hours at 37°C, allowing viable cells to convert the MTT reagent into insoluble purple formazan crystals.

Following incubation, the MTT-containing medium was carefully discarded without disturbing the crystals. The formazan crystals were dissolved by adding dimethyl sulfoxide (DMSO) (or acidified isopropanol where appropriate), and the plates were gently shaken until complete dissolution of the crystals occurred.[14]

The absorbance of each well was measured at 570 nm using a microplate ELISA reader. Cell viability was calculated by comparing the absorbance of treated cells with that of untreated control cells.[15]

The percentage of cell viability was calculated using the following equation:

The percentage of growth inhibition was determined using:

Morphological alterations such as cell shrinkage, rounding, membrane blebbing, reduced cell density, and loss of adherence were simultaneously examined under an inverted phase-contrast microscope. Since MCF-7 cells are adherent in nature, detached cells were carefully monitored during washing procedures to minimize experimental error. Minimal cell loss was observed, indicating reliable estimation of cell viability.[16,17]

MTT Assay Protocol for AGS Cells

Human gastric adenocarcinoma (AGS) cells were cultured under identical environmental conditions at 37°C with 5% CO_2 in complete growth medium. Cells were maintained in the logarithmic phase of growth before experimentation.[18]

AGS cells were seeded into sterile 96-well plates at an optimized density suitable for their comparatively faster proliferation rate. After overnight incubation, cells were exposed to different concentrations of Scirrhinum 200, while untreated cells served as the negative control and 5-Fluorouracil (5-FU) served as the positive control. A vehicle control containing 0.3% ethanol was included.[19]

Following 24 or 48 hours of treatment, MTT reagent was added to each well to achieve a final concentration of 0.5 mg/mL, and plates were incubated for 3 hours under standard culture conditions.[20]

The resulting formazan crystals were dissolved using DMSO, and absorbance was measured at 570 nm using a microplate reader. Cell viability and growth inhibition percentages were calculated using the same equations described for the MCF-7 cell line.[21]

Changes in cellular morphology including cell rounding, shrinkage, cytoplasmic condensation, and reduced monolayer integrity were documented microscopically and correlated with MTT assay findings.[22]

Outcome Analysis and Statistical Methods

All experiments were carried out in triplicate (n = 3) and repeated independently to ensure reproducibility. The optical density (OD) values obtained from the MTT assay were expressed as mean ± standard deviation (SD).[23]

The percentage of viable cells and percentage growth inhibition were calculated relative to untreated control cells. Dose-response curves were generated using GraphPad Prism software, and the half maximal inhibitory concentration (IC_{50}) values were determined by nonlinear regression analysis wherever applicable.[24]

Statistical analysis was performed using GraphPad Prism version 9.0 (or equivalent statistical software). Differences between two groups were analysed using the Student's unpaired t-test, whereas comparisons among multiple groups were performed using one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test using untreated control cells as the reference group.[25]

A p-value less than 0.05 ($p < 0.05$) was considered statistically significant. Experimental data were presented in the form of tables, dose-response curves, bar graphs, and representative photomicrographs illustrating the cytotoxic effect of Scirrhinum 200 on cancer cell lines.[26]

Materials and Methods for Apoptosis Analysis

Principle

Apoptosis was assessed using the Annexin V-Allophycocyanin (APC)/Propidium Iodide (PI) dual staining assay followed by flow cytometric analysis. This assay is based on the externalization of phosphatidylserine (PS) from the inner to the outer leaflet of the plasma

membrane, which is one of the earliest biochemical events during apoptosis.[26]

Annexin V is a calcium-dependent phospholipid-binding protein with high affinity for phosphatidylserine. During early apoptosis, phosphatidylserine becomes exposed on the outer surface of the cell membrane and binds specifically to fluorescently labelled Annexin V-APC. Propidium iodide (PI), a DNA-binding fluorescent dye, cannot penetrate intact cell membranes and therefore stains only cells with compromised membrane integrity, such as late apoptotic or necrotic cells.[27]

The combination of Annexin V-APC and PI enables discrimination among:

Annexin V− / PI−: Viable (healthy) cells
Annexin V+ / PI−: Early apoptotic cells
Annexin V+ / PI+: Late apoptotic cells
Annexin V− / PI+: Necrotic cells

Experimental Procedure

MCF-7 and AGS cells were seeded into culture plates and incubated overnight under standard culture conditions (37°C, 5% CO_2). Cells were subsequently treated with the selected concentration of Scirrhinum 200, while untreated cells served as the negative control. Where applicable, 5-Fluorouracil (5-FU) was included as the positive control.

Following the treatment period, both floating and adherent cells were collected to ensure complete recovery of apoptotic cells. Adherent cells were gently detached using trypsin-EDTA, combined with floating cells, and washed twice with cold phosphate-buffered saline (PBS).

Approximately 1×10^5 cells were resuspended in Annexin V binding buffer. Subsequently, Annexin V-APC and Propidium Iodide (PI) were added according to the manufacturer's protocol. The cell suspension was incubated for 15 minutes at room temperature in the dark to prevent photobleaching of the fluorescent dyes.

Following incubation, additional binding buffer was added, and samples were analysed immediately using a flow cytometer equipped with appropriate excitation and emission filters.

A minimum of 10,000 cellular events were acquired for each sample. Appropriate compensation controls and unstained controls were used to eliminate spectral overlap and ensure accurate gating.[28]

Data Acquisition and Analysis

Flow cytometric data were analysed using dedicated software (such as FlowJo or equivalent). Cell populations were represented on a two-dimensional dot plot based on Annexin V-APC fluorescence versus PI fluorescence.

The percentages of viable, early apoptotic, late apoptotic, and necrotic cells were calculated for each treatment group. The apoptotic effect of Scirrhinum 200 was determined by comparing treated samples with untreated controls.

The results were expressed as mean ± SD of three independent experiments. Statistical analysis was performed using one-way ANOVA followed by Dunnett's post hoc test, with $p < 0.05$ considered statistically significant.

Representative flow cytometry dot plots and quantitative bar graphs were generated to illustrate the induction of apoptosis by Scirrhinum 200 in MCF-7 and AGS cancer cell.

RESULTS

Effect of *Scirrhinum 200* on MCF-7 Breast Cancer Cells

According to the MTT analysis, *Scirrhinum 200* had an effect on decreasing the viability of the two cancer cell lines, indicating that it possesses potent anti-cancer properties. Although its cytotoxic effect was lower than that of the standard drug, *Scirrhinum 200* effectively inhibited cancer cell growth at higher concentrations. Microscopic observations and apoptosis studies further supported these findings by revealing characteristic features of apoptotic cell death. These results suggest that *Scirrhinum 200* possesses promising anticancer potential.

SR NO	SAMPLE CODE	Conc. (µg/ml)	OD			Mean	% Of Inhibition	% Of Viability	IC50 (µg/ml)
1	Control		1.482			-	-	-	-
2	Standard	20	0.916	0.915	0.916	0.915	38.25%	61.75%	
	(5,Fluorouracil)	40	0.732	0.729	0.734	0.731	50.67%	49.33%	
		60	0.507	0.504	0.502	0.504	65.99%	34.01%	39.33
		80	0.315	0.312	0.314	0.313	78.87%	21.13%	
		100	0.208	0.205	0.207	0.206	86.09%	13.91%	
3	Scirrhinum 200	20	1.263	1.269	1.261	1.264	14.71%	85.29%	
		40	1.101	1.103	1.108	1.104	25.51%	74.49%	
		60	0.867	0.862	0.867	0.865	41.63%	58.37%	
		80	0.736	0.730	0.739	0.735	50.40%	49.60%	
		100	0.519	0.512	0.517	0.516	65.18%	34.82%	79.60

Table No. 01- Effects of compound against MCF-7 Cell lines

Both 5-Fluorouracil and *Scirrhinum 200* exhibited concentration-dependent cytotoxicity against cancer cells. The standard drug showed greater potency, with an IC_{50} value of 39.33 µg/ml and 86.09% inhibition at 100 µg/ml. *Scirrhinum 200* demonstrated action with 65.18% inhibition at the same concentration.

Overall, *Scirrhinum 200* possesses notable cytotoxic potential against the tested cells; however, its efficacy is lower than the standard drug, suggesting comparatively weaker anticancer activity.

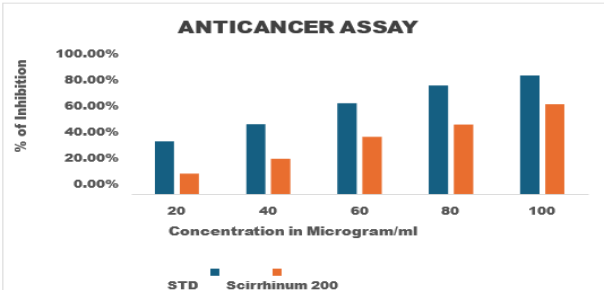


Fig. 1: Graphical analysis of concentration-dependent increase in anticancer activity for both the standard drug and *Scirrhinum 200.*

The graph shows a dose-dependent increase in anticancer activity for both the standard drug and *Scirrhinum 200*. As concentration increased, cell growth inhibition also increased. Although *Scirrhinum 200* was less potent than the standard drug, it demonstrated significant cytotoxicity against MCF-7 cells, achieving 65.18% inhibition at 100 µg/ml. These findings suggest that *Scirrhinum 200* possesses moderate anticancer potential and may inhibit cancer cell proliferation through apoptosis-related mechanisms.

Observations on Cell Morphology (Qualitative)

Dose-dependent changes in cell morphology were observed in MCF-7 cells treated with *Scirrhinum 200* using microscopy. The control cells showed normal morphology and the treatment cells showed progressive action with increasing concentrations. At higher concentrations, marked cell damage and detachment were observed, indicating cytotoxicity and apoptosis. These findings support the MTT assay results and suggest that *Scirrhinum 200* inhibits cancer cell growth through apoptosis-related mechanisms.

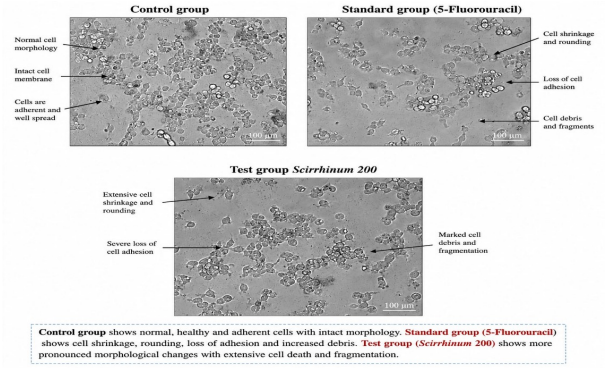
Effect of *Scirrhinum 200* on AGS Gastric Cancer Cells

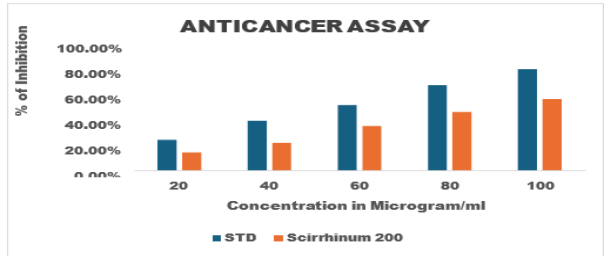
The MTT assay demonstrated that *Scirrhinum 200* produced a concentration-dependent reduction in the visibility AGS gastric cancer cells, achieving approximately 60% inhibition at 100 µg/mL. Although less potent than the standard drug, which showed about 85% inhibition, *Scirrhinum 200* exhibited significant cytotoxic activity. These findings suggest that *Scirrhinum 200* may suppress gastric cancer cell growth by cellular proliferation, supporting its potential as a candidate for further anticancer research

Table No. 02- Effects of test compound against AGS Cell line

The MTT assay results showed that both 5-Fluorouracil and *Scirrhinum 200* exhibited concentration-dependent cytotoxicity against AGS gastric cancer cells. The standard drug showed more potency with IC50 value of 55.46 µg/mL and 84.49% inhibition at 100 µg/mL. *Scirrhinum 200* showed moderate anticancer activity with 59.31% inhibition at 100 µg/mL and an IC50 value of 90.69 µg/mL. The findings of this study suggest that *Scirrhinum 200* can inhibit the growth and viability of AGS cells in a dose dependent manner, which may provide the basis for further anticancer investigations.

Fig. 2: Graphical analysis of concentration-dependent anticancer activity of *Scirrhinum 200* and the standard drug against AGS cell lines





The graph demonstrates a dose-dependent increase in anticancer activity for both *Scirrhinum 200* and the standard drug against AGS cells. As concentration increased, cell growth inhibition also increased. While the standard drug showed greater potency (84.49% inhibition at 100 µg/mL), *Scirrhinum 200* exhibited moderate cytotoxic activity with 59.31% inhibition, indicating its potential of inhibiting AGS cell growth in a concentration-dependent manner.

Observations on Cell Morphology (Qualitative)

Microscopic examination of AGS cells treated with *Scirrhinum 200* showed dose-dependent morphological changes. While control cell showed detachment and reduced cell density with increasing concentration. At higher concentrations, marked cell damage and increased floating cells were observed, indicating cytotoxicity and apoptosis. These findings support the MTT assay results and confirm the anticancer action of *Scirrhinum 200* on AGS cell growth and viability.

Comparative Analysis of Effects on MCF-7 vs. AGS

Comparative analysis showed that *Scirrhinum 200* exhibited dose-dependent anticancer activity against both cell lines. However, MCF-7 cells were more sensitive, with a lower IC_{50} value (79.60 µg/mL) and higher inhibition (65.18% at 100 µg/mL) examined with AGS cells (IC_{50} = 90.69 µg/mL; 59.31% inhibition). These findings suggest

that *Scirrhinum 200* has greater cytotoxic efficacy against breast cancer cells than gastric cancer cells.

Statistical Analysis

Statistical analysis showed that the cytotoxic effects of *Scirrhinum 200* were significant ($p < 0.05$) and concentration-dependent in both MCF-7 and AGS cell lines. Although 5-Fluorouracil exhibited stronger activity, *Scirrhinum 200* supporting its measurable anticancer potential, particularly against MCF-7 cells.

Apoptosis

It is studied through the Annexin V-FITC/PI test. The cancer cells were incubated with *Scirrhinum 200* for 48 h and then stained with Annexin V-FITC and Propidium Iodide and analyzed by flow cytometry. The test can differentiate between living, in terms cell membrane integrity, phosphatidylserine exposure. An elevated number of Annexin V-positive cells implies that apoptosis is being induced, thereby enabling the evaluation of the apoptosis-inducing capability of *Scirrhinum 200*, which is correlated with the results of the MTT test.

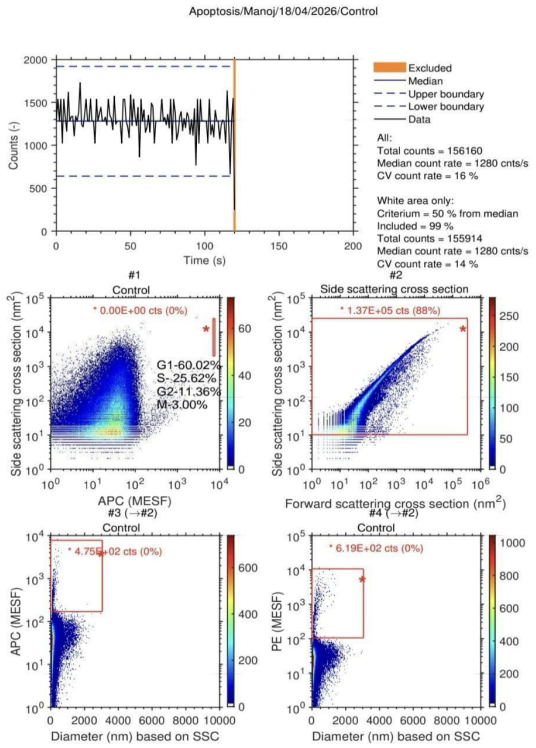


Fig. 3: Apoptosis Examination in Control Group

Interpretation of Apoptosis Examination in Control Group

Flow cytometric evaluation of the untreated control group demonstrated a predominantly healthy and viable cellular population with no major evidence of apoptosis or necrotic cell death. The cells exhibited a normal distribution with respect to forward scatter (FSC) and side scatter (SSC), indicating relatively normal cellular size and intracellular granularity. The absence of a prominent subpopulation with altered scatter characteristics further supports the maintenance of normal cellular morphology and integrity in the untreated control group.

Analysis of the cell-cycle distribution showed that the majority of cells were present in the G0/G1 phase, with additional populations distributed across the S and G2/M phases. The predominance of cells in the G0/G1 phase represents the normal proliferative status of the cell population, while the presence of cells in the S phase indicates ongoing DNA synthesis. The G2/M population represents cells progressing toward or undergoing mitosis. This distribution is consistent with an actively growing cell population under normal culture conditions.

The Annexin V/PI assay further confirmed the healthy status of the control cells. Annexin V-negative and PI-negative cells represented the major population, indicating intact plasma membranes and the absence of significant phosphatidylserine externalization. Since phosphatidylserine exposure is an early biochemical event during apoptosis, the low Annexin V-positive population suggests that spontaneous apoptosis was minimal in the untreated cells.

Similarly, the limited PI-positive population indicates that there was no substantial loss of plasma membrane integrity in the control group. PI is generally excluded by viable cells but enters cells with compromised membranes, making it useful for identifying late apoptotic or necrotic cells. The absence of a significant PI-positive population therefore indicates that spontaneous necrosis or extensive late-stage cell death was not evident under the experimental conditions.

The control group is particularly important for interpreting the effects of Scirrhinum 200 because it provides the baseline cellular response in the absence of treatment. A low basal level of apoptosis and necrosis in the control population ensures that any subsequent changes observed following Scirrhinum 200 treatment can be more appropriately attributed to the treatment rather than to spontaneous cell death, poor culture conditions, handling-related damage, or experimental variability.

The relatively normal cell-cycle profile of the control group also provides an essential reference for assessing whether Scirrhinum 200 alters cell-cycle progression. Changes in the proportion of cells in G0/G1, S, or G2/M phases following treatment can be compared with this untreated baseline to determine whether the test sample produces cell-cycle arrest, altered proliferation, or redistribution of cells between different phases of the cell cycle.

Overall, the flow cytometric findings in the untreated control group demonstrate a predominantly viable and proliferating cell population with minimal basal apoptosis and necrosis. The low Annexin V and PI positivity indicates preservation of cellular membrane integrity and the absence of significant spontaneous apoptotic or necrotic events. These findings establish a stable and reliable baseline against which the cellular effects of Scirrhinum 200 can be evaluated.

Furthermore, the healthy status of the control population strengthens the interpretation of the subsequent treatment findings. Since the control cells did not exhibit substantial apoptosis or necrosis, any increase in Annexin V-positive or PI-positive cells in the Scirrhinum 200-treated group would provide evidence of treatment-associated cell death. Conversely, if the treated cells show a profile comparable to the control group, it would suggest that the treatment did

not substantially induce apoptosis or necrosis under the tested experimental conditions.

Thus, the control group serves as an essential internal reference for validating the flow cytometric experiment and provides a physiological baseline for assessing treatment-induced changes in cell viability, apoptosis, necrosis, and cell-cycle progression.

Fig. 4: Apoptosis Analysis in *Scirrhinum 200* Treated Cell

Interpretation of Apoptosis Analysis in *Scirrhinum 200* Treated Cell

Flow cytometric analysis was performed to investigate whether treatment with Scirrhinum 200 induced apoptotic or necrotic cell death in the experimental cell population. The analysis was based on Annexin V and propidium iodide (PI) staining, which allows discrimination between viable cells, early apoptotic cells, late apoptotic/necrotic cells, and dead cells. Annexin V binds to phosphatidylserine exposed on the outer surface of the plasma membrane during the early stages of apoptosis, whereas PI enters cells with compromised plasma membrane integrity and therefore identifies late apoptotic or necrotic cells.

Following treatment with Scirrhinum 200, the majority of cells remained within the viable cell population, indicating that the treatment did not produce substantial loss of cell viability under the experimental conditions employed. The relatively low proportion of Annexin V-positive cells suggests that Scirrhinum 200 did not markedly activate the phosphatidylserine externalization process associated with early apoptosis. Similarly, the absence of a considerable PI-positive population indicates that extensive membrane damage, late apoptosis, or necrotic cell death was not evident.

The flow cytometric findings therefore suggest that Scirrhinum 200 did not induce a measurable apoptotic response at the tested concentration and exposure period. This observation is important because an anticancer effect, if present, may not necessarily be mediated through classical apoptosis. The biological response may involve alternative mechanisms such as inhibition of cellular proliferation, cell-cycle modulation, metabolic alterations, autophagy, oxidative stress, senescence, or other non-apoptotic pathways. Consequently, the absence of a prominent Annexin V/PI response should not be interpreted as definitive evidence of complete absence of biological activity.

The cell-cycle profile further provides information regarding the effect of Scirrhinum 200 on cellular proliferation. The distribution of cells across the G0/G1, S, and G2/M phases indicates that a substantial proportion of the treated cells continued to progress through the cell cycle. The presence of cells within the S phase indicates ongoing DNA synthesis and cellular proliferation. Therefore, under the present experimental conditions, Scirrhinum 200 did not produce a pronounced accumulation of cells in a particular cell-cycle phase that could be directly associated with strong cell-cycle arrest.

When considered together, the apoptosis and cell-cycle findings indicate that Scirrhinum 200, at the concentration and exposure duration investigated, did not produce substantial apoptotic or necrotic cell death. The observed cellular response may therefore be characterized as limited or non-apoptotic under the tested conditions. These findings should be interpreted in conjunction with other experimental observations, such as MTT cytotoxicity results, morphological assessment, nuclear staining, and other mechanistic assays, rather than being considered in isolation.

It is also important to recognize that apoptosis is a time- and concentration-dependent biological process. A single concentration and exposure period may not be sufficient to detect the maximum apoptotic response. The absence of significant Annexin V/PI positivity could therefore be related to the selected dose, duration of treatment, cellular model, or sensitivity of the assay. Some forms of cellular death may also occur through pathways that are not efficiently captured by Annexin V/PI staining.

Further investigations using a broader concentration range and multiple exposure periods would help establish whether Scirrhinum 200 produces a concentration- or time-dependent apoptotic response. Additional mechanistic studies involving caspase-3, caspase-8, caspase-9, Bax, Bcl-2, mitochondrial membrane potential, cytochrome-c release, PARP cleavage, and related apoptotic markers could provide more definitive evidence regarding involvement of intrinsic or extrinsic apoptotic pathways. Assessment of autophagy-related markers, reactive oxygen species, DNA damage, and cellular senescence may also help identify alternative mechanisms responsible for the observed biological effects.

Overall, the present flow cytometric findings indicate that Scirrhinum 200 did not induce substantial measurable apoptosis or necrosis in the tested cells under the experimental conditions. The predominance of viable cells and the limited Annexin V/PI positivity suggest that classical apoptotic cell death was not the major cellular response at the investigated concentration and exposure time. Nevertheless, these findings do not exclude the possibility of subtle, delayed, concentration-dependent, or apoptosis-independent biological effects. Therefore, comprehensive mechanistic investigations using different concentrations, treatment durations, and molecular markers are warranted to clarify the cellular mechanism of action of Scirrhinum 200.

DISCUSSION

Cancer is a complex and multifactorial disease requiring therapeutic strategies that can effectively control malignant cell proliferation while minimizing toxicity to normal tissues. Although conventional approaches such as chemotherapy, radiotherapy, surgery, targeted therapy, and immunotherapy have substantially improved cancer management, treatment-related toxicity, resistance, recurrence, and disease progression remain important challenges. The present study therefore investigated the in-vitro cytotoxic potential of *Scirrhinum 200* against MCF-7 breast cancer and AGS gastric cancer cells.

The present findings demonstrated that *Scirrhinum 200* produced a concentration-dependent reduction in cell viability in both MCF-7 and AGS cell lines. The IC_{50} values obtained were 79.60 µg/mL for MCF-7 cells and 90.69

µg/mL for AGS cells. At the highest tested concentration of 100 µg/mL, inhibition of 65.18% and 59.31% was observed in MCF-7 and AGS cells, respectively. These findings indicate that the tested preparation affected the viability and growth of both cancer cell models under the experimental conditions employed.

Microscopic examination further supported the MTT findings. Treatment resulted in morphological changes such as cell shrinkage, rounding, detachment from the culture surface, and reduced cell density. Such alterations are commonly observed during cellular injury and loss of viability. However, morphological observations alone cannot establish a specific mechanism of cell death; therefore, additional mechanistic investigations are necessary.

Comparison with Previous Studies

Scirrhinum has historically been described in homoeopathic literature as a nosode associated with scirrhous cancer and breast-related conditions. Historical materia-medica literature describes its use in cases of breast tumours and other cancer-associated conditions. However, such historical reports are based primarily on clinical observations and traditional homoeopathic practice rather than controlled laboratory experiments.

Materia Medica

Published literature concerning homoeopathic preparations and cancer has reported investigations involving different cancer cell lines and proposed mechanisms such as inhibition of cellular proliferation, apoptosis, and cell-cycle modulation. However, the available evidence remains heterogeneous, and there is comparatively limited experimental evidence specifically evaluating *Scirrhinum 200* against both breast and gastric cancer cell lines.

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In this context, the present study provides preliminary laboratory-based evidence regarding the effect of *Scirrhinum 200* on MCF-7 and AGS cells. The use of two distinct cancer cell lines is an important feature because it permits preliminary comparison of the response of breast and gastric cancer models. Nevertheless, the differences observed between MCF-7 and AGS cells should be interpreted cautiously because cellular sensitivity can vary according to cell-line characteristics and experimental conditions.

Comparison with Previously Published Homoeopathic Cancer Cases

Previous clinical reports have described the use of *Scirrhinum 200* as part of homoeopathic management in patients with different types of cancer. For example, published case material by Ramakrishnan and colleagues describes cases involving prostate, breast, cervical, and other cancers in which *Scirrhinum* was administered along with other homoeopathic medicines. Some of these reports described clinical or radiological improvement during treatment.

These reports differ substantially from the present investigation. Published case reports represent individual clinical observations, often involving multiple medicines and individualized treatment protocols, whereas the present study evaluated a defined experimental preparation against cancer cell lines under controlled in-vitro conditions. Therefore, the clinical observations reported previously cannot be directly equated with the cytotoxic activity observed in the present study.

A further important difference is that some published cases used *Scirrhinum* in combination or alternation with other homoeopathic medicines. For example, reports have described *Scirrhinum 200* being administered alternately with constitutional or organ-specific remedies in patients with breast or prostate cancer. Consequently, the response reported in such cases cannot be attributed exclusively to *Scirrhinum 200*. In contrast, the present study specifically evaluated the effect of *Scirrhinum 200* on MCF-7 and AGS cells.

CONCLUSION

The present study demonstrated dose dependent anticancer activity of *Scirrhinum 200* against MCF-7 breast cancer and AGS gastric cancer cell lines. MCF-7 cells were more sensitive to treatment (IC_{50} = 79.60 µg/mL) than AGS cells (IC_{50} = 90.69 µg/mL). The microscopic observations showed cell shrinkage, rounding, detachment and reduced cell density, as seen in the cytotoxic effects of the MTT assay. Flow cytometric analysis of MCF-7 cells showed increased apoptosis (6.86% vs. 3.00% in control) and accumulation of cells in the S phase, indicating cell cycle arrest and antiproliferative activity. Although less potent than 5-Fluorouracil, *Scirrhinum 200* demonstrated moderate anticancer potential...

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