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Original Article

GI_{50} Inhibition Study Using Sulforhodamine B (SRB) Assay Method on Breast Cancer Cell Line (MCF-7) Using Ethanolic Extracts of Leaves of *Chromolaena odorata* and *Capparis moonii*

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ABSTRACT

The study titled "GI_{50} inhibition study using Sulforhodamine B (SRB) assay method on breast cancer cell line (MCF-7) using ethanolic extracts of leaves of *Chromolaena odorata* and *Capparis moonii*" investigates the anticancer potential of leaf extracts. **Objectives:** The research focuses on assessing the effects of ethanolic extracts of leaves of both plants and their combination using the Sulforhodamine B assay. **Materials and Methods:** These extracts of selected plants' leaves were prepared by maceration technique using ethanol as a solvent. Using the SRB assay, the extracts were tested *in vitro* against cell lines at certain doses. Adriamycin was used as a standard drug. **Results:** The extracts were found to contain alkaloids, flavonoids, and phenolic compounds. While *Capparis moonii* showed limited efficacy, *Chromolaena odorata* demonstrated significant inhibitory activity with a GI_{50} value of 17 µg/mL, indicating its potential as an anticancer agent. **Conclusion:** The study suggests the anticancer potential of *Chromolaena odorata* on the MCF-7 cell line, meets the National Cancer Institute's criteria for effectiveness, whereas *Capparis moonii* requires further optimization for better results. This study would direct the investigation into the precise chemical components of the extracts that exhibit anticancer activity, the possibility of synergistic effects when plant extracts are combined, and the potential for experimental modifications to increase the effectiveness of plant extracts.

Keywords: Anti-Cancer, *Capparis moonii*, *Chromolaena odorata*, GI_{50} inhibition, MCF-7, Cancer cell line study, Sulforhodamine B (SRB) assay.

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INTRODUCTION

Even after decades of clinical research, as well as trials, the cancer disease remains a primary reason of death.[1] An idea that the immune system controls cancer growth is returning after a century of debate. Extensive evidence from animal models and persuasive evidence from human patients suggest that there is, in fact, a functional cancer immunosurveillance pathway that functions as an extrinsic tumor suppressor.[2,3]

Breast cancer is difficult to avoid because it develops in a multiple-step process. The identification of breast cancer stem cells reveals the pathophysiology and tumor drug-resistant processes, and numerous genes linked to breast cancer are discovered.[4-7] The breast cancer claimed almost 570,000 lives in 2015.[8] Cell lines are a suitable candidate for this type of work since they may be used widely in numerous areas of research in laboratories, especially as *in vitro* models for cancer research. Basic research is necessary for better treatment. MCF-7 is utilized cell line for breast cancer that has been supported by numerous research groups for more than 40 years.[9]

In this study, the substances were tested *in vitro* using the Sulforhodamine B (SRB) assay method, a popular and accurate way to measure anti-proliferative activity. Amino xanthene dye SRB is a vivid pink colour that has two sulfonic groups. Trichloroacetic acid (TCA) is used to fix the cells. In mildly acidic environments, the anionic protein stain proteins' basic amino acid residues are bound by SRB electrostatically. Still, TCA permits both binding and solubilization of dye, which is regulated by pH changes. Because the colour is long-lasting, it makes it possible to easily test performance, and it is soluble for optical density and may be quantitatively removed from the cell's measurements.[10-12]

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Plants contain phytochemicals that can impede the growth of cancer since the process of carcinogenesis involves multiple signaling pathways. Phytochemicals function as multitargeted agents and are strong anticancer agents. According to published research, particular plants, such as *Chromolaena odorata* and *Capparis moonii*, are abundant in phytochemicals of many different kinds, including flavonoids and phenols, which are also known to have anticancer properties.[13-15]

Among the most popular human breast cancer cell lines in research, especially for investigations and treatment of breast cancer, is this cell line.

A subtype, known as estrogen receptor-positive breast carcinoma is distinguished by the presence of estrogen receptors on the tumor cells' surface. MCF-7 cells are commonly used to simulate this kind of breast cancer. Although it can occur in later stages, this subtype is frequently linked to breast cancer which is in its early stages.[16,17]

MATERIALS AND METHODS

Collection, Authentication, and Extraction of Plant

The leaves of *Chromolaena odorata* were collected from Borivali National Park, Mumbai, and authenticated by Agarkar Research Institute, Pune. The leaves of *Capparis moonii* were collected from Sangli, Maharashtra, and authenticated by Dr. Harshad Pandit, Ph.D. Botany Mumbai. The leaves of the plants -*Chromolaena odorata* and *Capparis moonii* were extracted by maceration using ethanol as solvent. Bioactive chemicals are frequently extracted from plant leaves using ethanol as a solvent. This approach is frequently used in plant chemistry, pharmacology, and herbal medicine; **Polarity:** Because it is a polar solvent, ethanol can extract a variety of substances, such as phenolics, alkaloids, flavonoids, and essential oils. Because ethanol may dissolve both polar and slightly non-polar molecules, these chemicals are frequently soluble in it.

Efficiency: Ethanol is a popular option for a variety of extractions, including methanol, water, and hexane, since it effectively extracts a wide range of bioactive chemicals from plant material. Both hydrophilic (soluble in water) and lipophilic (soluble in fat) substances can be dissolved by it, though the effectiveness may differ based on the kind of plant.

Safety: Ethanol is less hazardous and generally safer than other organic solvents like ether or chloroform, particularly in food or medicinal applications. In comparison to other solvents, it is also thought to be more environmentally friendly.[18]

The extracts were stored for anticancer studies.

Preliminary phytochemical screening

The extracts underwent initial phytochemical screening following a practical handbook by C.K. Kokate.[19]

In vitro - anticancer activity using SRB assay

The investigation occurred at the ACTREC at the Tata Memorial Centre in Kharghar, Navi Mumbai. The MCF-7 cell line was used in the *in vitro* investigation.

Procedure

The Table 1 demonstrates the procedure for the SRB assay.[10]

Activity Criteria

Inhibitory concentration GI_{50} for synthetic formulations ≤10µg/mL.

For natural products or plant extracts, GI_{50}≤20 µg/mL.[20,21]

Positive control: Adriamycin drug was used as a standard to ensure the experimental set was working.

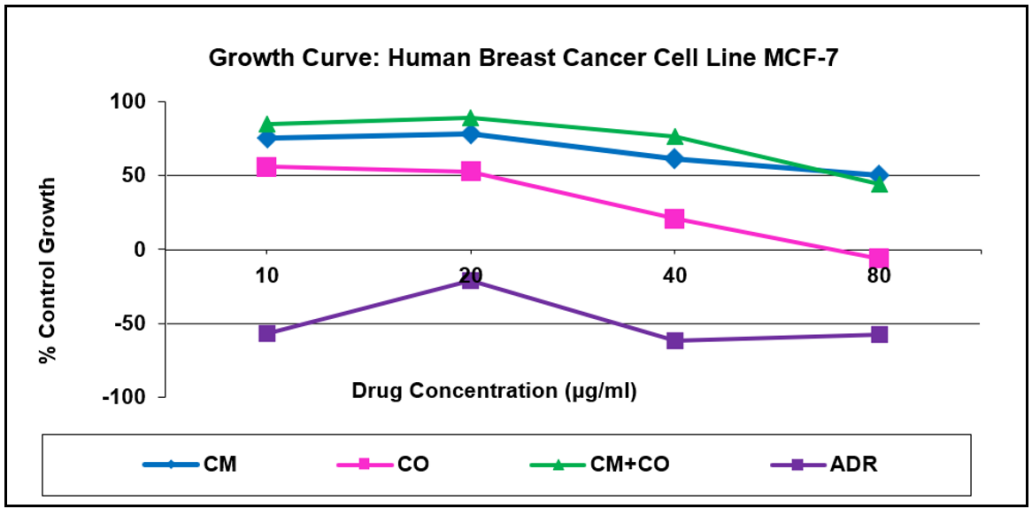

Figure 1: The figure indicates extracts showing inhibition of percent growth in a dose-dependent manner - MCF-7.[7]

Table 1: **The table demonstrates the procedure for the SRB assay.**

Step	Description
Cell Culturing	Cultivate cell line in a suitable medium augmented; 2 mM L-glutamine and fetal bovine serum (10%)[20-23]
Cell Seeding	Seed 100 µL of 5000 cells/well onto 96-well microtiter plates.
Pre-Drug Incubation	Incubate plates for 1 day at 37°C, 5% CO_2, 95% air, and 100% RH.
Drug Preparation	-Freeze and thaw experimental drugs. -Dissolve in the appropriate solvent at 100 mg/mL. - Dilute to 1 mg/mL with water.
Drug Dilution	Dilute the 1 mg/mL stock with medium to 100 µg/mL, 200 µg/mL, 400 µg/mL, and 800 µg/mL at the time of administration.
Drug Administration	Add 10 µL aliquots of drug dilution to wells containing 90 µL of medium (final volume: 100 µL).
Post-Drug Incubation	Incubate the plates for 48 hr under normal conditions (37°C, 5% CO_2, 95% air, 100% humidity).
Termination of Experiment	50 µL of cold 30% TCA to the wells. Incubate for 60 min at 4°C.
Washing	Dispose of the supernatant and wash the plates five times with tap water.
Sulforhodamine B Staining	Add 50 µL of 0.4% Sulforhodamine B in 1% acetic acid. Incubate for 20 min at room temperature.
Dye Removal	Wash the wells 5 times with 1% acetic acid to remove dye (unbound)
Air Dry	Allow plates to air dry
Dye Elution	Elute with 10 mM Trizma base (bound dye)
Absorbance Measurement	Measure absorbance at 540 nm with a reference wavelength of 690 nm using a plate reader.
Data Analysis	Calculate percentage growth using: (Average Absorbance of Test Wells / Average Absorbance of Control Wells) × 100.

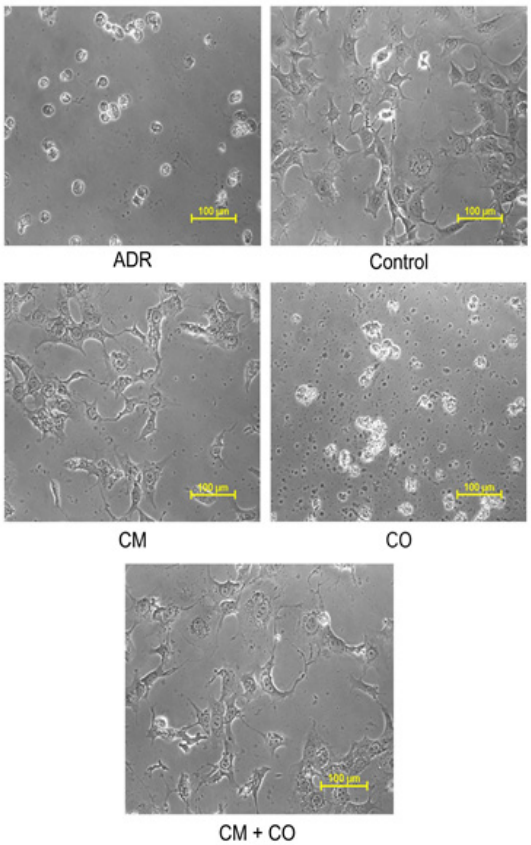

Figure 2: The figure indicates the morphology of MCF-7 when treated with std. Adriamycin, Control, and ethanolic extracts of CM, CO, and CM+CO.[7]

Table 2: **The results in Table demonstrates percent control growth of human breast cancer cell line in the presence of ethanolic extract of CM, CO, CM+CO, and std. Adriamycin.[7]**

Drug Concentrations (µg/mL)	10.00	20.00	40.00	80.00
***Capparis moonii* (CM)**				
Experiment 1	70.5	70.0	52.0	47.1
Experiment 2	75.7	76.9	62.7	48.6
Experiment 3	80.4	88.1	69.9	55.2
Average Values	75.5	78.3	61.5	50.3
***Chromolaena odorata* (CO)**				
Experiment 1	52.8	50.3	18.7	-6.7
Experiment 2	61.9	54.9	21.6	-8.7
Experiment 3	52.8	51.8	21.8	-3.2
Average Values	55.8	52.4	20.7	-6.2
CM+CO				
Experiment 1	76.1	85.5	74.7	46.7
Experiment 2	91.9	87.9	82.2	47.6
Experiment 3	86.6	93.7	72.0	38.9
Average Values	84.9	89.0	76.3	44.4
Adriamycin (ADR)				
Experiment 1	-67.5	-23.5	-63.4	-40.3
Experiment 2	-67.3	-4.7	-71.4	-61.0
Experiment 3	-36.0	-34.2	-50.3	-71.6
Average Values	-56.9	-20.8	-61.7	-57.6

Table 3: **The results in Table demonstrates the Median Growth Inhibition (GI_{50}) for ethanolic extracts of CM, CO, CM+CO, and std. Adriamycin.[7]**

Drug concentrations calculated from the graph (µg/mL)		
Cell line	Name of Drug	GI_{50}*
MCF-7	CM	78
	CO	17
	CM+CO	75
	Adriamycin	<10

RESULTS AND DISCUSSION

Phytochemical screening and analytical study

The ethanolic extract of leaves of *Capparis moonii* and *Chromolaena odorata* states the presence of alkaloids, carbohydrates, glycosides, flavonoids, and phenolic compounds. The TLC reveals a similar retention factor for the standard Rutin and Quercetin with the sample extracts.[22]

Anticancer activity

Anticancer activity of ethanolic extracts of leaves *Chromolaena odorata, Capparis moonii*, and their combination was screened for human breast cancer cell line at the con. of 10, 20, 40, 80 µg/mL. Figure 1 indicates extracts showing inhibition of percent growth in a dose-dependent manner - MCF-7. Table 2 demonstrates the percent control growth of human breast cancer cell line in the presence of ethanolic extract of CM, CO, CM+CO, and standard Adriamycin, while Table 3 demonstrates the Median Growth Inhibition (GI_{50}) for ethanolic extracts of CM, CO, CM+CO, and standard Adriamycin.

Figure 2 indicates the morphology of MCF-7 when treated with standard Adriamycin, Control, and ethanolic extracts of CM, CO, and CM+CO.

The National Cancer Institute (NCI) states that $GI_{50} \leq 20$ µg/mL is regarded as indicating activity for natural compounds or plant extracts. Extracts were determined to be ineffective against the breast and colon cancer cell line based on the established criteria. Nevertheless, scientific research on these plant extracts clarified and found the presence of several chemical components with anti-cancer potential, demonstrating the abundance of anti-cancer phytoconstituents found in plants. Using this taking into account, additional studies on optimizing experimental conditions for a chosen cell, and a review of the literature line is necessary, which could increase the likelihood that some cancer treatment candidates will be successful.[23,24]

Given the richness of phenolic or flavonoid content in these plants, researchers can use these extracts to create innovative drug

delivery methods, such as liposomes, metallic nanoparticles, and nanoemulsions, to augment the medicinal effects of these plants.

CONCLUSION

Using the Sulforhodamine B (SRB) assay, the screening experiment effectively assessed the experimental drug's impact on cell proliferation. The study's findings show that the drug had dose-dependent cytotoxicity, with cell viability decreasing as drug concentrations increased. The medication effectively inhibits cell proliferation at higher concentrations, with considerable growth inhibition seen at 400 µg/mL and 800 µg/mL, according to the estimated percentage growth in the test wells compared to control wells.

Through the measurement of cellular protein concentration, the SRB assay offered a trustworthy and precise indicator of cell viability after treatment. The medication exerts strong cytotoxic effects on the tested cell line, as evidenced by the observed decrease in absorbance at higher drug concentrations. To evaluate the drug's mode of action, selectivity for malignant versus normal cells, and potential therapeutic index, more research is necessary.

In summary, the investigational medication exhibits significant effects on cell growth inhibition, suggesting that it may be a promising therapeutic treatment. To better understand the drug's efficacy and safety profile, future research should focus on improving its formulation, investigating its pharmacokinetics, and carrying out *in vivo* investigations.

The extracts of given plants inhibited the percent control growth in dose-dependent character, for cancer cell lines. GI_{50} concentration against the extract of leaf of *Capparis moonii* and its combination with *Chromolaena odorata* did not provide satisfactory results but the leaf extracts of *Chromolaena odorata* embodied triumphant results for anticancer activity on human breast cancer cell line according to the NCI acceptance criteria.

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ABBREVIATIONS

SRB: Sulforhodamine B; **CM:** *Capparis moonii*; **CO:** *Chromolaena odorata*; **ACDSF:** Anti-Cancer Drug Screening Facility; **GI:** Growth Inhibition; **ADR:** Adriamycin; **TCA:** Trichloroacetic acid; **NCI:** National Cancer Institute; **mM:** Millimolar; **mg:** Milligram; **mL:** Milliliter; **RH:** Relative Humidity; **ACTREC:** Advanced Centre for Treatment, Research, and Education in Cancer.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

- GI_{50} Inhibition Study Using Sulforhodamine B (SRB) Assay Method on Breast Cancer Cell Line (MCF-7) Using Ethanolic Extracts of Leaves of *Chromolaena odorata* and *Capparis moonii*.
- To evaluate the anticancer potential of ethanolic extracts from the leaves of *Chromolaena odorata* and *Capparis moonii* against the MCF-7 breast cancer cell line using the Sulforhodamine B (SRB) assay.
- Leaves of *Chromolaena odorata* were collected from Borivali National Park, Mumbai, and *Capparis moonii* from Sangli, Maharashtra.
- Extracts were prepared using ethanol via the maceration technique.
- Presence of alkaloids, flavonoids, phenolic compounds, glycosides, and carbohydrates was confirmed.
- MCF-7 cells were cultured and treated with different concentrations (10, 20, 40, and 80 µg/mL) of plant extracts.
- Adriamycin was used as a standard positive control.
- The GI_{50} (concentration required to inhibit 50% growth) was determined.
- *Chromolaena odorata* exhibited significant anticancer activity with a GI_{50} value of 17 µg/mL, meeting the National Cancer Institute (NCI) criteria for effectiveness ($GI_{50} \leq 20$ µg/mL for natural compounds).
- *Capparis moonii* showed limited efficacy with a GI_{50} of 78 µg/mL.
- A combination of both extracts (CM+CO) did not significantly enhance the anticancer effect (GI_{50}=75 µg/mL).
- Adriamycin, the standard drug, showed a GI_{50} of <10 µg/mL.
- *Chromolaena odorata* demonstrates potential as an anticancer agent against MCF-7 cells.
- *Capparis moonii* requires further optimization for better efficacy.
- Future research should explore the specific active compounds, their mechanisms, and novel drug delivery methods to enhance effectiveness.

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