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Testing the GI50 of human lung cancer cells (A-549) with Sulforhodamine B (SRB) assay method using ethanolic extracts of Chromolaena odorata and Capparis moonii leaves

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ABSTRACT

The study investigates the *in-vitro* anticancer potential of ethanolic extracts from the leaves of *Chromolaena odorata* and *Capparis moonii* using the Sulforhodamine B (SRB) assay on the lung cancer cell line (A-549). The extracts of selected plants' leaves were prepared using the Soxhlet apparatus technique using ethanol as a solvent. These plants were thought to have strong anti-cancer effects because they were abundant in phytochemicals like flavonoids and phenolic compounds. Alkaloids, flavonoids, and phenolic substances were present in the extracts. *Chromolaena odorata* leaves extract had a GI_{50} of 44 µg/ml for lung cancer cell lines, whereas *Capparis moonii* leaves extract had a GI_{50} of over 80 µg/ml. When both extracts are combined, the GI_{50} concentration is greater than 80 µg/ml. Given the presence of phenolic as well as flavonoid components in these plants, improving their healing qualities and lowering the GI_{50} concentration to the appropriate level need either optimizing experimental settings or converting extracts into novel drug delivery methods.

Keywords: *A-549 human lung cancer cell line, Sulforhodamine B (SRB) assay, Chromolaena odorata, Capparis moonii, GI_{50} inhibition, anticancer.*

1. INTRODUCTION

Despite years of research and testing new treatments, cancer remains a top cause of illness and mortality [1]. After a century of discussion, there is a resurgence in the belief that the immune system regulates cancer growth. There is strong evidence from both animal studies and human patients indicating the presence of a functional cancer immunosurveillance pathway acting as an external tumor suppressor. [2-4]

Lung cancer, with around 1.2 million new cases in 2000, ranks as the most prevalent cancer globally, accounting for 12.3% of all cancer cases. Many instances of lung cancer are identified when a symptom associated with the primary, metastatic illness, or paraneoplastic syndrome manifests. Lung cancers originate from a series of molecular and morphological changes that start in histologically normal epithelium. [5-7] Researching using cell lines, such as A-549 for lung cancer studies, typically yields more precise results in experiments. [8-10]

This study assessed substances *in vitro* utilizing the Sulforhodamine B (SRB) assay method, a widely used and precise approach to evaluate anti-proliferative effects. The amino xanthene dye SRB is a bright pink hue with 2 sulfonic groups. Trichloroacetic acid (TCA) is employed for cell fixation. In slightly acidic conditions, SRB binds electrostatically to the basic amino acid residues of proteins stained with anionic protein. However, TCA allows for the dye to both bind and solubilize, with regulation dependent on pH fluctuations. Due to its enduring colour, it allows for straightforward performance evaluation, is soluble for optical density, and can be precisely extracted from the cell's readings. [11-14]

Phytochemicals found in plants can hinder cancer growth by targeting various signaling pathways involved in carcinogenesis. Phytochemicals act on multiple targets and possess potent anticancer properties. Information from research publications shows that certain plants, like *Chromolaena odorata* and *Capparis moonii*, contain a variety of phytochemicals, such as flavonoids and phenols, that are recognized for their anti-cancer properties. [15,16]

Capparis moonii W. was historically used in the diagnosis of cough and asthma. Antihistamine is considered a useful agent for the treatment of allergies. The family – Capparidaceae, comprises essential medicinal properties proven to be immensely used as a remedy in traditional medicinal systems. The plant is perennial and is generally found in India's Western Ghats region. It is used as an antioxidant, laxative, anti-diabetic, anti-hyperuricemia, and even hepatoprotective. The pharmacological investigations also prove different parts of the CAP species to be medicinal. [17-19]

Chromolaena odorata (Asteraceae) commonly known as Siamese weed is a primary weed. A medicinal plant found in tropical Asia, Australia and West Africa. The use of *Chromolaena odorata* is documented in established systems such as Siddha, Unani, and Ayurveda. The pharmacological properties of this plant are quite different. It was extracted from *Chromolaena odorata*. It has a wide variety of attractive but limited compounds, and its pharmacological activity is assorted. [20-24]

2. METHODS AND MATERIALS

Collection, authentication, and extraction of plant:

Fig. 1: Leaves, dried powder, and extraction of leaves

(A) – Leaves of *Capparis moonii* , (B) – Powder of leaves of *Capparis moonii*, (C) -Ethanolic extract of leaves of *Capparis moonii* , (D) - Leaves of *Chromolaena odorata*, (E) - Powder of leaves of *Chromolaena odorata*, (F) - Ethanolic extract of leaves of *Chromolaena odorata*

Chromolaena odorata leaves from Borivali National Park, Mumbai, was verified by Agarkar Research Institute, Pune. *Capparis moonii* leaves were gathered in Sangli, Maharashtra, and verified by Dr. Harshad Pandit, Ph.D. (botany), in Mumbai. The extraction of the leaves of *Chromolaena odorata* and *Capparis moonii* was done through the Soxhlet apparatus with ethanol as the solvent. The samples were kept for research on cancer treatment.

Preliminary phytochemical screening:

The samples were subjected to an initial phytochemical screening following CK Kokate's guidelines.[25]

Analytical studies:

TLC and HPTLC analyses were used to determine the chemical components present in the extracts. The study was conducted at Gahlot Institute of Pharmacy, Navi Mumbai, and Geo Chem Laboratories Pvt. Ltd. respectively.

***In-vitro* anticancer activity using SRB assay**:

The task was conducted at ATREC, Kharghar, Navi Mumbai, a part of Tata Memorial Centre, focused on cancer treatment, research, and education. The research was carried out in a cell line, A-549, in a laboratory setting.

Procedure: [21,22,26,27]

The cell lines were grown in an appropriate medium containing 10% fetal bovine serum and 2 mm l-glutamine. For the present screening experiment, 5000 cells/well were inoculated into 96 well microtiter plates in 100 µl. After cell inoculation, the microtiter plates were incubated at 37° c, 5 % CO_2, 95 % air, and 100 % relative humidity for 24 hours before the addition of experimental drugs.

Experimental drugs were solubilized in an appropriate solvent at 100mg/ml diluted to 1mg/ml using water and stored frozen before use. At the time of drug addition, an aliquot of frozen concentrate (1mg/ml) was thawed and diluted to 100 µg/ml, 200 µg/ml, 400 µg/ml, and 800 µg/ml with a complete medium containing test article.

10 µl portions of various drug dilutions were introduced into the corresponding microtiter wells along with 90 µl of a medium, creating final drug concentrations of 10 µg/ml, 20 µg/ml, 40 µg/ml, and 80 µg/ml, as needed. After compound addition, plates were incubated at standard conditions for 48 hours and the assay was terminated by the addition of cold TCA.

Cells were fixed in situ by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°c.

The liquid on top was thrown away and the plates were rinsed with tap water five times before being left to dry in the air. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1 % acetic acid. The plates were air-dried. The bound stain was subsequently eluted with a 10 mm trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with a 690 nm reference wavelength. The growth percentage was determined for each test well compared to the control well on a plate-to-plate basis. Percent growth was expressed as the ratio of the average absorbance of the test well to the average absorbance of the control wells multiplied by 100. The percentage growth was determined at each drug concentration level using six absorbance measurements. Growth inhibition of 50 % (GI_{50}) drug concentration resulted in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. Values were calculated for all the three parameters. If the level of activity was reached; however, if the effect was not reached or was exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested.

Activity criteria: [28]

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

For natural products or plant extracts GI_{50} ≤ 20 µg/ml.

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

3. RESULT AND DISCUSSION

Phytochemical screening and analytical study:

The ethanolic extract of the leaves of *Capparis moonii* (CM) and *Chromolaena odorata* (CO) contained alkaloids, carbohydrates, glycosides, flavonoids, and phenolic substances. When compared to the sample extracts, the TLC shows that quercetin and rutin have comparable retention factors. The presence of rutin and quercetin in the supplied leaf extracts is confirmed by HPTLC, which displays threshold peaks.

Anticancer activity:

The potential of ethanolic extracts from CM, CO, and CM+CO leaves to inhibit cancer was tested on human lung cancer cells (A-549) using concentrations of 10, 20, 40, and 80 (µg/ml). The National Cancer Institute (NCI) considers a GI_{50} value of ≤ 20 µg/ml to show effectiveness for natural compounds or plant extracts. Extracts were found to be inefficient against the breast and colon cancer cell line according to the set standards. However, studies on these plant extracts have revealed the existence of multiple chemical compounds with anti-cancer properties, indicating the rich variety of anti-cancer phytoconstituents present in plants. Considering this, further research is needed to optimize experimental conditions for a specific cell type and to review existing literature, ultimately improving the chances of success for potential cancer treatments. [28-35]

Human Lung Cancer Cell Line A-549				
% Control Growth				
Drug Concentrations (µg/mL)	**10.00**	**20.00**	**40.00**	**80.00**
***Capparis moonii* (CM)**				
Experiment 1	95.5	83.0	82.2	85.5
Experiment 2	92.6	90.5	84.8	75.0
Experiment 3	98.2	84.8	83.2	77.4
Average Values	95.4	86.1	83.4	79.3
***Chromolaena odorata* (CO)**				
Experiment 1	93.5	79.7	43.7	9.9
Experiment 2	92.1	85.5	43.7	7.1
Experiment 3	90.6	86.7	49.4	13.4

Average Values	92.1	83.9	45.6	10.1
CM+CO				
Experiment 1	91.7	91.9	88.6	81.8
Experiment 2	91.3	89.2	88.6	72.2
Experiment 3	100.3	97.3	88.9	77.4
Average Values	94.4	92.8	88.7	77.1
ADR				
Experiment 1	-14.9	-14.7	-34.3	-52.5
Experiment 2	-23.9	-21.9	-44.4	-60.7
Experiment 3	-19.6	-8.0	-35.5	-53.7
Average Values	-19.5	-14.9	-38.0	-55.6

Table 1: Percent control growth of human lung cancer cell line in the presence of ethanolic extract of CM, CO, CM+CO, and std. Adriamycin.

Drug concentrations (µg/ml) calculated from graph		
Cell line	Drug	GI_{50}
A-549	CM	>80
	CO	44
	CM+CO	>80
	ADR	<10

Table 2: Median growth inhibition (GI_{50}) for ethanolic extracts of CM, CO, CM+CO, and std. Adriamycin.

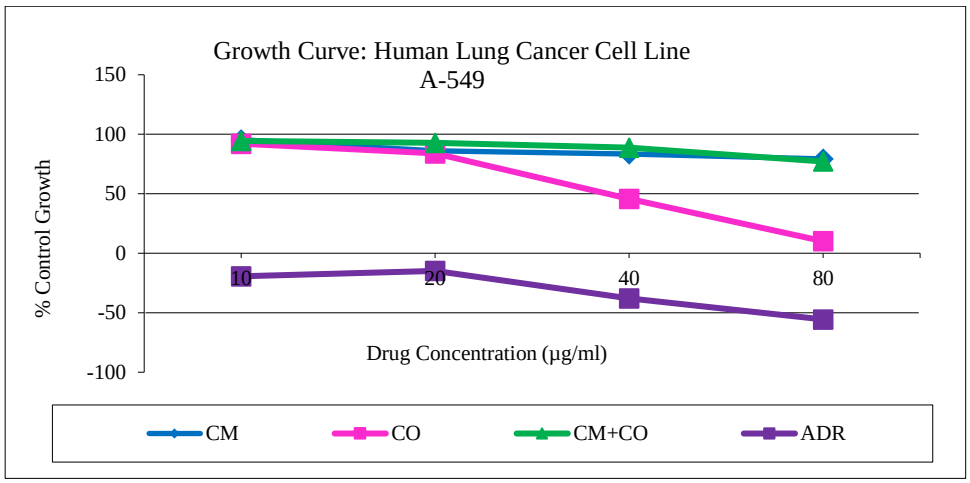

Fig. 2: Extracts showing inhibition of percent growth in a dose-dependent manner.

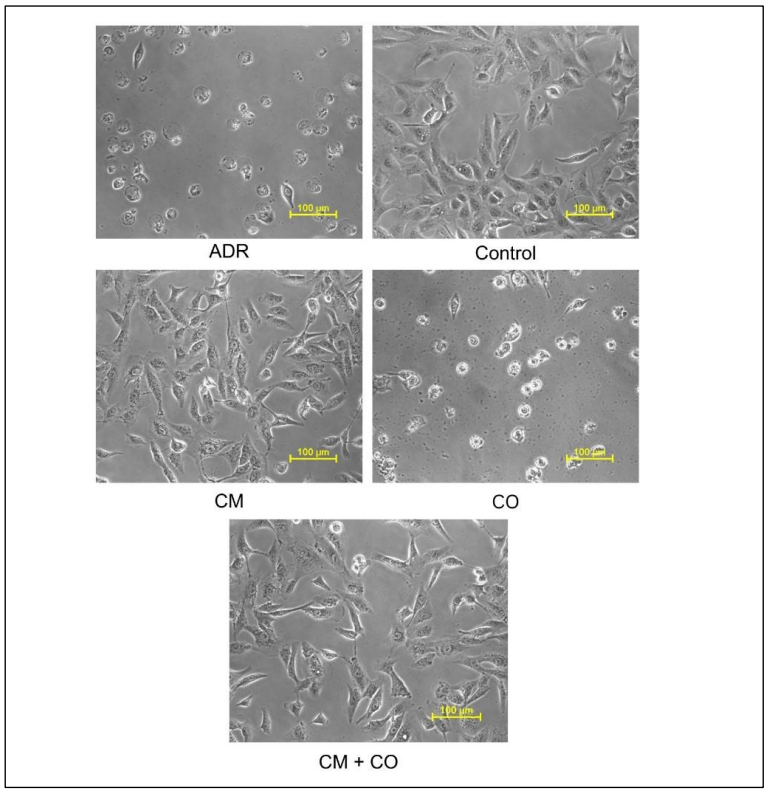

Fig. 3: Morphology of A-549 when treated with std. Adriamycin, control, and CM, CO, and CM+CO ethanolic extracts.

4. CONCLUSION

The plant extracts inhibited the growth control percentage in a manner dependent on the dose. The leaf extracts of *Capparis moonii* and *Chromolaena odorata* had negligible anticancer activity against the A-549 human lung cancer cell line, as indicated under GI_{50} concentration by the National Cancer Institute's criteria for acceptance. These findings emphasize the importance of refining experimental parameters and investigating sophisticated drug delivery methods, like nanoparticles or liposomal formulations, to improve the bioavailability and therapeutic effectiveness of these plant-derived substances. Future studies ought to concentrate on utilizing the phytochemical characteristics of these plants to discover more effective derivatives or synergistic blends for cancer treatment.

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Conflict of interest: None

Abbreviation

CM – *Capparis moonii,* CO – *Chromolaena odorata,* mM – millimolar, µg/ml – microgram per millilitre, nm – nanometre.

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