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Gi50 Inhibition Study Using Sulforhodamine B (Srb) Assay Method On Mouse Melanoma Cell Line (B16-F10), Using Ethanolic Extracts Of Leaves Of Chromolaena Odorata And Capparis Moonii.

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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 mouse melanoma cell line (B16-F10). These plants, rich in phytochemicals such as flavonoids and phenolic compounds, were hypothesized to possess significant anti-cancer properties. Extracts were prepared using Soxhlet apparatus and tested across multiple concentrations (10–80 µg/ml). Results demonstrated that the extract did not achieve the inhibitory concentration threshold (GI50 ≤ 20 µg/ml) defined by the National Cancer Institute for natural compounds. Morphological analysis and dose-response studies confirmed suboptimal inhibition of melanoma cell growth compared to the standard Adriamycin. While the extracts displayed a dose-dependent response, they were not effective in inhibiting melanoma growth. The findings suggest the need for enhanced experimental conditions and novel drug delivery systems to harness the therapeutic potential of these phytochemical-rich plants..

Keywords: *Chromolaena odorata, Capparis moonii, Sulforhodamine B, Adriamycin, mouse melanoma, anticancer.*

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1. INTRODUCTION

Cancer continues to be a leading cause of morbidity and death even after decades of basic and clinical research as well as trials of promising new treatments.[1] The impact of cancer immunotherapy on clinical cancer care is snowballing.[2]

The idea that the immune system controls the growth of cancer is making a comeback 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.[3,4]

One of the best illustrations of the interaction between genetics and environment in the pathophysiology of cancer is found in melanoma. Benign collections of melanocytes known as melanocytic nevi (moles) have received particular interest as possible precursor lesions. More industrialized nations have seen a surge in melanoma incidence over the past 50 years than any other cancer form. Normal pigment cells called melanocytes, which are produced from the neural crest and found on the basement membrane of epithelial surfaces, are the source of melanoma.[5,6]

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. Aminoxanthene dye SRB is a vivid pink color 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 color 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.[7-9]

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.[10]

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 Capparis species to be medicinal.[11-13]

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 traditional systems. 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.[14-18]

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*

The leaves of *Capparis moonii* were collected from Sangli, Maharashtra, and authenticated by Dr. Harshad Pandit, Ph.D. (Botany). The leaves of *Chromolaena odorata* were collected from Borivali National Park, Mumbai, and authenticated by Agarkar Research Institute, Pune. The leaves of the plants –*Chromolaena odorata* and *Capparis moonii* were extracted using Soxhlet apparatus using ethanol as a solvent. The extracts were stored for anticancer studies.

Preliminary phytochemical screening:

The extracts underwent initial phytochemical screening following C.K. Kokate.[19]

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

The activity was carried out at Tata Memorial Centre – Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Kharghar, Navi Mumbai. The study was conducted *in vitro* in cell line - B16-F10.

Procedure:[20-24]

The cell lines were cultured in a suitable medium that included 10% fetal bovine serum and 2 mM L-glutamine. In the current screening experiment, 5000 cells/well were introduced into 96-well microtiter plates with a volume of 100 µL.

Following cell inoculation, the microtiter plates were kept at 37 °C with 5 % CO_2, 95 % air, and 100 % relative humidity for 24 hours before the introduction of experimental drugs.

Experimental medications were dissolved in a suitable solvent at a concentration of 100mg/ml, then diluted to 1mg/ml with water and kept frozen until needed. When the drug was added, a portion of the frozen concentrate (1mg/ml) was thawed and diluted to concentrations of 100 µg/ml, 200 µg/ml, 400 µg/ml, and 800 µg/ml using a complete medium that included the test article. Aliquots of 10 µl from these various drug dilutions were introduced into the suitable microtiter wells that already contained 90 µl of the medium, achieving the desired final drug concentrations, namely 10 µg/ml, 20 µg/ml, 40 µg/ml, and 80 µg/ml.

Following the addition of the compound, plates were incubated under standard conditions for 48 hours, and the assay was concluded by the addition of cold TCA. Cells were fixed in place by slowly adding 50 µl of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was removed; the plates were rinsed five times with tap water and allowed to air-dry. Sulforhodamine B (SRB) solution (50 µl) at zero. 4 % (w/v) in 1 % acetic acid was applied to each of the wells, and the plates were kept at room temperature for 20 minutes. Following the staining process, any unbound dye was collected, and the remaining dye was eliminated by rinsing five times with 1% acetic acid. The dishes were left to dry in the air. The attached stain was then eluted using a 10 mM trizma base, and the absorbance was measured on a plate reader at a wavelength of 540 nm, using a 690 nm reference wavelength.

Percent growth was determined on a plate-by-plate basis for test wells in comparison to control wells. Percent Growth was defined as the ratio of the mean absorbance from the test well to the mean absorbance from the control wells, multiplied by 100.

Activity Criteria: [25]

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 CM and CO leaves showed the presence of alkaloids, carbohydrates, glycosides, flavonoids, and phenolic substances. Consequently, the overall makeup and levels of phenolic compounds indicated that the selected plan ts have the potential to inspire new studies aimed at gaining a deeper understanding of their possible effects on human health in the scientific community. The presence of rutin and quercetin in the provided leaf extracts is validated by HPTLC, which shows distinct peaks.

Anticancer activity:

The anticancer potential of ethanolic extracts from leaves CM, CO, and CM+CO was evaluated B16-10 at concentrations of 10, 20, 40, and 80 µg/ml.

The National Cancer Institute (NCI) indicates that a GI_{50} of 20 µg/ml or lower is considered a sign of efficacy for natural compounds or plant extracts. Extracts were found to be ineffective against the breast and colon cancer cell lines according to the established criteria. Nonetheless, scientific studi es on these plant extracts elucidated and discovered various chemical constituents with anti-cancer properties, highlighting the richness of anti-cancer phytochemicals present in plants. Considering this, further research on enhancing experimental conditions for a selected cell, along with a literature review, is essential to boost the chances of success for certain cancer treatment candidates.

Due to the abundant phenolic or flavonoid content present in these plants, scientists can utilize these extracts to develop novel drug delivery systems, including liposomes, metallic nanoparticles, and nanoemulsions, to enhance the therapeutic benefits of these plants.

Mouse Melanoma Cell Line B16-F10

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

% Control Growth				
Drug Concentrations (µg/ml)	10.00	20.00	40.00	80.00
Capparis moonii				
Experiment 1	88.3	103.8	91.3	84.3
Experiment 2	77.2	106.7	83.5	77.3
Experiment 3	86.0	106.7	83.0	68.7
Average Values	83.8	105.7	86.0	76.8
Chromolaena odorata				
Experiment 1	70.8	77.9	44.4	14.4
Experiment 2	83.3	83.2	42.4	10.6
Experiment 3	81.1	81.8	38.3	3.3
Average Values	78.4	81.0	41.7	9.5
CM+CO				
Experiment 1	90.3	112.2	105.4	82.5
Experiment 2	92.6	106.4	94.8	61.7
Experiment 3	81.2	94.5	90.4	57.7
Average Values	88.0	104.3	96.9	67.3
Adriamycin				
Experiment 1	-87.7	-88.2	-81.1	-33.9
Experiment 2	-88.5	-88.4	-86.8	-52.1
Experiment 3	-90.9	-90.6	-88.9	-45.8
Average Values	-89.1	-89.1	-85.6	-43.9

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

Drug concentrations (µg/ml) calculated from graph		
Cell line	**Name of Drug**	**GI_{50}***
B16-F10	*Capparis moonii*	>80
	Chromolaena odorata	40
	CM+CO	>80
	Adriamycin	<10

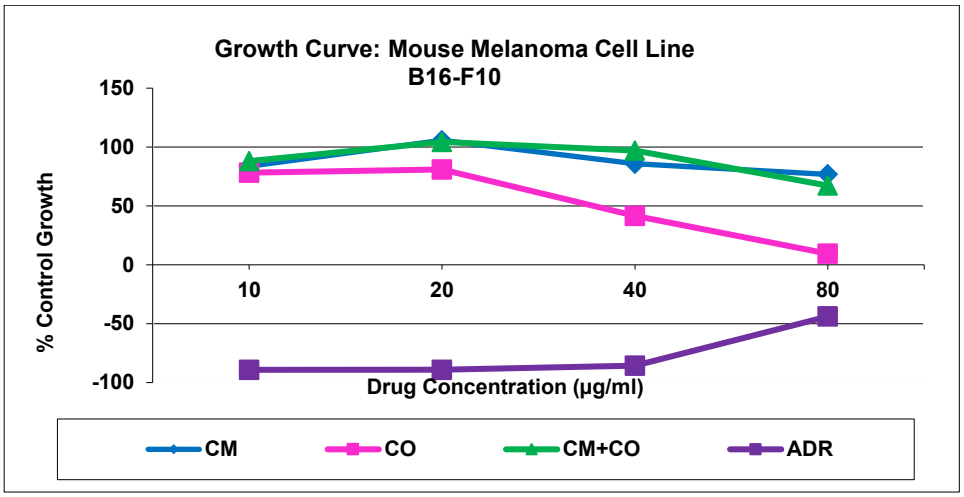

Fig. 2: Extracts showing inhibition of percent growth in a dose-dependent manner – B16-F10.

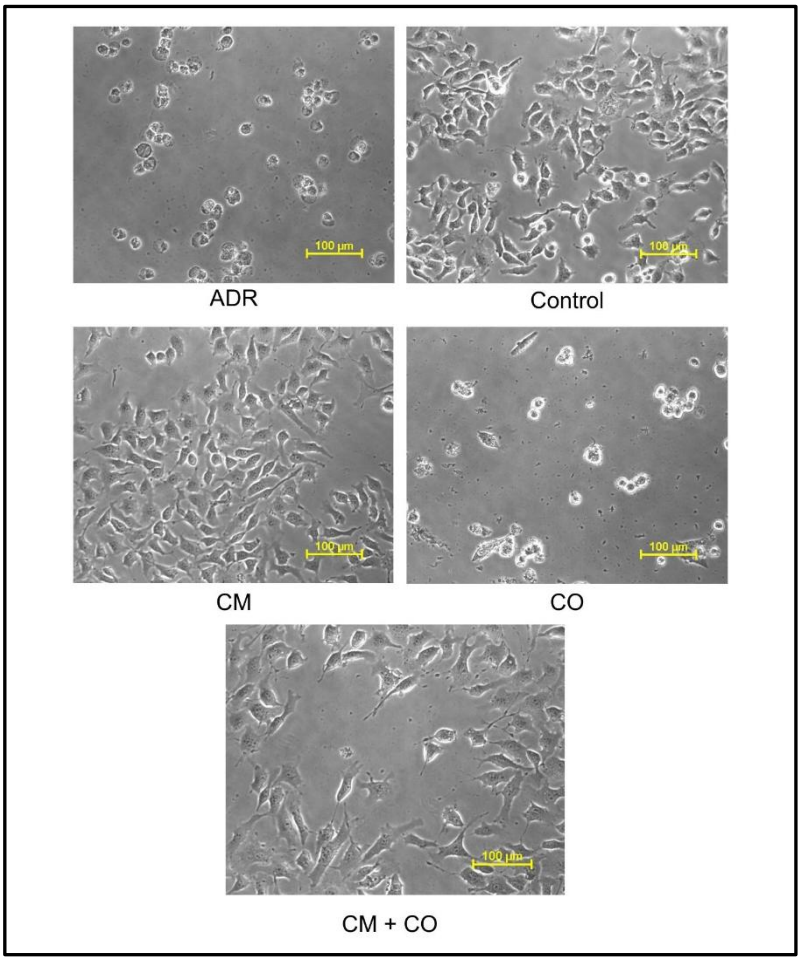

Fig. 3: Morphology of B16-F10 when treated with std. Adriamycin, Control, and ethanolic extracts of CM, CO, and CM+CO.

CONCLUSION

The results of this research emphasize the possible yet restricted anticancer effects of ethanolic extracts from Chromolaena odorata and Capparis moonii leaves against the B16-F10 mouse melanoma cell line. Despite their richness in phytochemicals such as flavonoids and phenolic compounds, the extracts did not reach the inhibitory concentration threshold ($GI_{50} \leq 20$ µg/ml) established by the National Cancer Institute. Morphological analysis and dose-response examinations indicate that although the extracts demonstrate a certain level of dose-dependent inhibition, their effectiveness

is inferior to that of standard Adriamycin.

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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