World Journal of Pharmaceutical Science and Research
www.wjpsronline.com
Research Article
ISSN: 2583-6579
SJIF Impact Factor: 3.454
Year - 2023
Volume: 2; Issue: 6
Page: 254-262

GI50 INHIBITION STUDY ON ETHANOLIC EXTRACTS OF *CONVOLVULUS PLURICAULIS*, *MICHELIA CHAMPACA* AND *CHROMOLAENA ODORATA* AGAINST HUMAN BREAST (MCF-7) AND COLON CANCER (HT-29) CELL LINE BY SULFORHODAMINE B (SRB) ASSAY METHOD

Manjushree Pawar[1*] and Jayesh Dwivedi[2]

[1*]Registered PhD scholar, Department of Pharmaceutical Sciences, Pacific Academy of Higher Education and Research University, Udaipur-313024, Rajasthan, India.

[2]Professor and HOD, Department of Pharmaceutics, Pacific Academy of Higher Education and Research University, Udaipur-313024, Rajasthan, India.

Article Received: 02 October 2023 | | ***Article Revised: 22 October 2023*** | | ***Article Accepted: 29 November 2023***

Corresponding Author: Manjushree Pawar
Registered PhD scholar, Department of Pharmaceutical Sciences, Pacific Academy of Higher Education and Research University, Udaipur-313024, Rajasthan, India.

ABSTRACT

Background: Cancer immunology is a rapidly growing field of research that studies interactions between the immune system and cancer cells. The selected plants have proven their immunomodulatory effects via cellular and humoral mediated immunity in animal models in our previous research work. **Objective:** To screen the ethanolic extracts of *Convolvulus pluricaulis* (CP), *Michelia champaca* (MC), *Chromolaena odorata* (CO) and combination of above three plants (CP+MC+CO) for *in-vitro* anticancer activity using breast and colon human cancer cell lines - MCF-7 and HT-29. **Methods and materials:** The extracts of selected plants (whole plant extract) were prepared by using maceration technique using ethanol as a solvent. The extracts were subjected for *in-vitro* anticancer potential using sulforhodamine B (SRB) assay against breast and colon cancer cell lines at concentrations of 10, 20, 40 and 80 µg/ml. The standard drug Adriamycin was used. **Results:** All the extracts inhibited percent control growth in dose dependent manner for both breast and colon cancer cell line. GI50 (Concentration of drug causing 50% inhibition of cell growth) for breast cancer cell line in CP extract was more than 80 µg/ml, and in MC, CO, and CP+MC+CO extract it was found to be 77.6, 49.5 and 78.2 µg/ml. GI50 for colon cancer cell line in all the extracts was found to be more than 80 µg/ml. Optimization in experimental conditions or turning extracts into novel drug delivery systems can enhance the medicinal effects of the plants and can reduce the GI50 concentration. **Conclusion:** Optimization in experimental conditions or turning extracts into novel drug delivery systems can enhance the medicinal effects of the plants and can reduce the GI50 concentration to desired standard level considering the phenolic/flavonoid richness of these plants.

KEYWORDS: Percent control growth, GI50, Sulforhodamine B, *Convolvulus pluricaulis*, *Michelia champaca*, *Chromolaena odorata*.

INTRODUCTION

Cancer is the secondary immunodeficiency disorder, defined as a disease in which abnormal cells group grow in uncontrolled manner without considering the cell division rules. By 2040, number of new cancer cases per year is expected to rise to 29.5 million and number of cancer related deaths is expected to be 16.4 million. Figure 1 and 2 shows the estimated number of new cancer cases and number of cancer deaths in 2020 – for all age groups and both sexes. As per the globocan cancer statistics, breast cancer is in first category and colon cancer in third category of most frequently diagnosed cancers.[1-4]

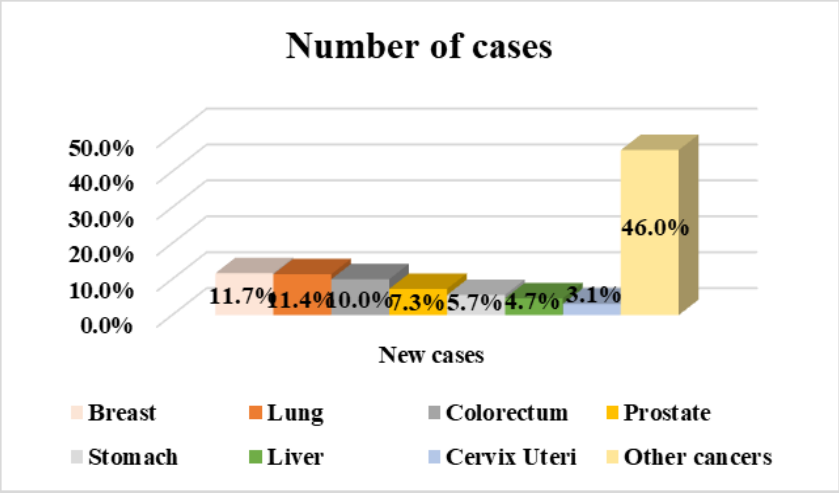

Figure 1: Estimated number of new cases in, for both sexes, all ages in 2020.

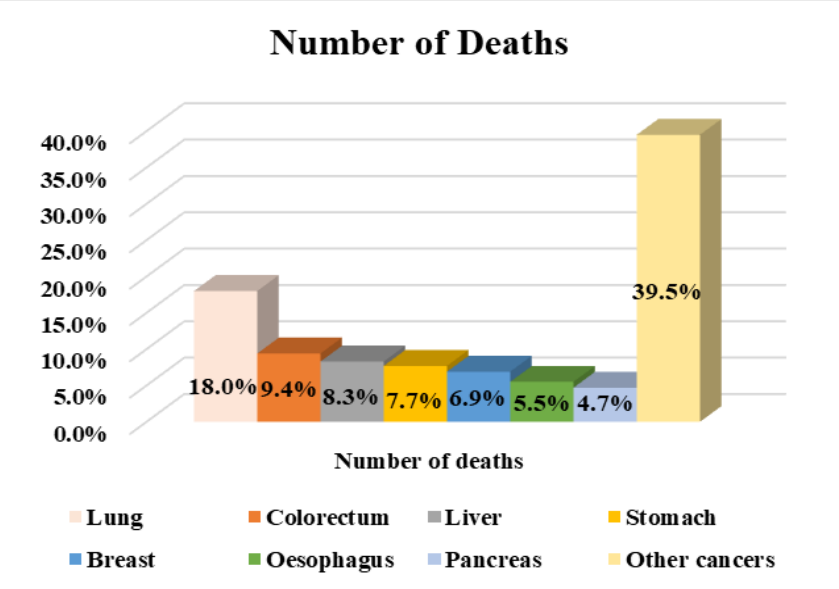

Figure 2: Estimated number of deaths, for both sexes, all ages, in 2020.

Breast cancers are tumors starting in epithelial cells that lines organs and tissues, it spreads to nearby lymph nodes and spread further to other organs. From the epithelial cells of the breast, molecularly distinct tumors originate.[1,5] MCF-7 – a human breast cancer cell line can capture the molecular characteristics and study heterogeneity of tumors. HT 29 – human colon cancer cell line is used to assess the anticancer effects of different drugs and it can study the structural and

molecular events in cell differentiation. Literature states that these cell lines can express the characteristics of mature intestinal cells such as enterocytes or mucus producing cells.[6]

Sulforhodamine B (SRB) assay method was used in this research work to test the samples *in-vitro* as it is widely used and sensitive method for testing anti-proliferative activity. SRB is a bright pink aminoxanthene dye with two sulfonic groups. The cells are fixed with trichloroacetic acid (TCA) and under mild acidic conditions, the anionic protein stain of SRB binds electrostatically to basic amino acid residues of proteins, whereas, TCA allows binding and solubilisation of dye which can be controlled by variations in pH. The dye is stable for a long period of time thus enabling the ease of assay performance and can be extracted quantitatively from the cells and solubilized for optical density measurements.[7-9]

Phytochemicals found in plants have the ability to suppress the development of cancer as carcinogenesis is multifactorial with various signalling pathways. Phytochemicals act as multitargeted agents which represent convincing anticancer compound.[10] Literature revealed that selected plants *Convolvulus Pluricaulis*, *Michelia Champaca*, and *Chromolaena Odorata* are rich in phytochemicals belonging to various classes specially flavonoids/phenols wherein this class of compounds are known to exhibit the anticancer potential. Also in our previous research, the above mentioned plants have reported to show presence of various phenolic compounds. Further, these plants possess immunomodulatory potential and have shown effects on cellular and humoral immunity in our previous work. This encouraged us to select these plants for *in-vitro* anticancer study.

METHODS AND MATERIALS

Collection, Authentication and Extraction of Plant

The plants were collected from Sanjay Gandhi National Park, India and authenticated at Agharkar Research Institute, Pune, India, by Dr. R. K. Chaudhary. The whole plant extract was prepared by maceration method using ethanol as a solvent. These extracts were stored at 8-15°C for anticancer studies.

Preliminary phytochemical screening

The extracts were subjected for preliminary phytochemical screening as described in C.K. Kokate.[11]

Analytical studies

The extracts were subjected for GC-MS and LC-MS analysis for the identification of chemical constituents. The work was conducted at IIT Bombay.

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* on two cell lines MCF-7 and HT-29.

Procedure: Cell lines were grown in appropriate medium containing 10% fetal bovine serum and 2 mM L-glutamine. For 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 h prior to addition of experimental drugs. Experimental drugs were solubilized in appropriate solvent at 100 mg/ml and diluted to 1mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquote of frozen concentrate (1mg/ml) was thawed and diluted to 100 µg/ml, 200 µg/ml, 400 µg/ml, and 800 µg/ml with complete medium

containing test article. Aliquots of 10 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µl of medium, resulting in the required final drug concentrations – 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml. After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA (trichloroacetic acid). 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 supernatant was discarded; the plates were washed five times with tap water and air dried. 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. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells * 100. Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as: [Ti/C]×100%.[7-9,12,13]

Activity Criteria: Inhibitory concentration GI50 for synthetic compounds/formulations ≤ 10µg/ml. For natural products or plant extracts GI50 ≤ 20 µg/ml.

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

RESULTS AND DISCUSSION

Phytochemical screening and analytical study

The ethanolic extract of CP, MC and CO revealed the presence of phenolic compounds and tannins, flavonoids, alkaloids, and carbohydrates. In our previous research work of GC-MS and LC-MS analysis, ethanolic extracts of CP, MC and CO revealed the presence of various chemical constituents belonging to class of phenols - flavonoids, terpenes, long chain fatty acids, alkaloids, glucosinolate. In LC-MS analysis, phenolic compounds – flavonoids and phenylpropanoids were identified in all the three ethanolic extracts of CP, MC, and CO.[14,15] Thus, the comprehensive composition and content of phenolic compounds showed that the selected plants can encourage scientific community to address new studies to understand their potential on human health.

Anticancer activity

Anticancer activity of ethanolic extracts of CP, MC, CO, and CP+MC+CO were screened for human breast cancer cell line (MCF-7) and human colon cancer cell line (HT-29) at the concentrations of 10, 20, 40 and 80 (µg/ml). All the extracts inhibited percent control growth in dose dependent manner for both breast and colon cancer cell line [Figure 3 and 4]. The effect is more at 80 µg/ml [Table 1 and Table 3]. GI50 concentration against breast cancer cell line for CP extract was found to be >80µg/ml, for MC, CO, and CP+MC+CO extract it was found to be 77.6, 49.5, and 78.2 µg/ml [Table 2]. GI5O concentration against colon cancer cell line for all the extracts was found to be >80 µg/ml [Table 4]. The cell morphology for MCF-7 and HT-29 is shown in Figure 5 and 6.

As per the National Cancer Institute (NCI), for natural products or plant extracts, GI50 ≤ 20 µg/ml is considered to show the activity. Following the criteria, extracts were found to be unsatisfactory against the breast and colon cancer cell line. However, analytical studies of these plant extracts elucidated and identified presence of many chemical constituents having anti-cancer potential which shows that plants are rich in anticancer phytoconstituents. Taking this into consideration, further literature review and research on optimization of experimental parameters for selected cell line is required, which may help to improve the success rates of selected cancer drug candidates.[16] To enhance the medicinal effects of these plants extracts, researchers can turn these plant extracts to novel drug delivery systems such as nano emulsions, metallic nanoparticles, liposomes etc. considering the phenolic or flavonoids content richness of these plants.

Table 1: Percent control growth of breast cancer cell line in presence of ethanolic extract of CP, MC, CO, CP+MC+CO and standard Adriamycin.

Human breast cancer cell line MCF-7				
% Control Growth				
Drug concentrations (µg/ml)	**10**	**20**	**40**	**80**
CP				
Experiment 1	102.7	101.8	100.8	83.0
Experiment 2	95.4	98.2	94.3	89.7
Experiment 3	102.3	106.2	96.9	85.3
Average values	100.1	102.1	97.3	86.0
MC				
Experiment 1	86.2	86.9	72.2	51.5
Experiment 2	77.3	79.1	63.7	47.6
Experiment 3	84.9	88.4	71.2	46.4
Average values	82.8	84.8	69.0	48.5
CO				
Experiment 1	81.0	81.3	61.4	22.0
Experiment 2	72.3	77.0	56.0	26.5
Experiment 3	76.1	83.6	55.1	24.4
Average values	76.5	80.6	57.5	24.3
CP+MC+CO				
Experiment 1	85.3	94.3	78.7	46.9
Experiment 2	75.8	84.4	71.2	47.6
Experiment 3	77.5	87.4	71.4	45.7
Average values	79.5	88.7	73.8	46.7
ADR				
Experiment 1	-82.9	-85.5	-83.7	-84.5
Experiment 2	-79.6	-78.9	-84.8	-80.4
Experiment 3	-83.0	-79.9	-87.3	-82.2
Average values	-81.9	-81.4	-85.2	-82.4

Table 2: Median growth inhibition (GI50) for ethanolic extracts of CP, MC, CO, CP+MC+CO and standard Adriamycin.

Drug concentrations (µg/ml)		
Cell line	**Name of Drug**	**GI50**
MCF-7	Ethanolic extract of CP	>80
	Ethanolic extract of MC	77.6
	Ethanolic extract of CO	49.5
	Combination - CP+MC+CO	78.2
	Adriamycin (standard)	<10

Table 3: Percent control growth of colon cancer cell line in presence of ethanolic extract of CP, MC, CO, CP+MC+CO and standard Adriamycin.

Human colon cancer cell line HT-29				
% Control growth				
Drug concentrations (µg/ml)	**10**	**20**	**40**	**80**
CP				
Experiment 1	127.5	124.4	118.4	108.5
Experiment 2	111.6	112.3	105.1	106.6
Experiment 3	109.6	105.7	106.6	108.0
Average values	116.2	114.1	110.0	107.7
MC				
Experiment 1	124.7	114.9	113.0	92.9
Experiment 2	108.1	105.9	100.7	94.4
Experiment 3	110.8	100.8	102.8	94.2
Average values	114.5	107.2	105.5	93.8
CO				
Experiment 1	124.3	117.5	98.6	49.9
Experiment 2	114.1	111.5	89.1	65.2
Experiment 3	112.4	105.5	97.7	67.0
Average values	116.9	111.5	95.1	60.7
CP+MC+CO				
Experiment 1	120.2	119.4	114.1	81.2
Experiment 2	106.8	111.0	102.7	87.8
Experiment 3	108.7	101.6	104.6	89.8
Average values	111.9	110.7	107.2	86.3
ADR				
Experiment 1	-2.3	-0.4	0.4	1.3
Experiment 2	1.1	1.2	1.1	1.8
Experiment 3	1.9	3.0	3.1	3.0
Average values	0.2	1.3	1.5	2.0

Table 4: Median growth inhibition (GI50) for ethanolic extracts of CP, MC, CO, and CP+MC+CO and standard Adriamycin.

Drug concentrations (µg/ml)		
Cell line	**Name of drug**	**GI50**
HT-29	Ethanolic extract of CP	>80
	Ethanolic extract of MC	>80
	Ethanolic extract of CO	>80
	Combination (CP+MC+CO)	>80
	Adriamycin (standard)	<10

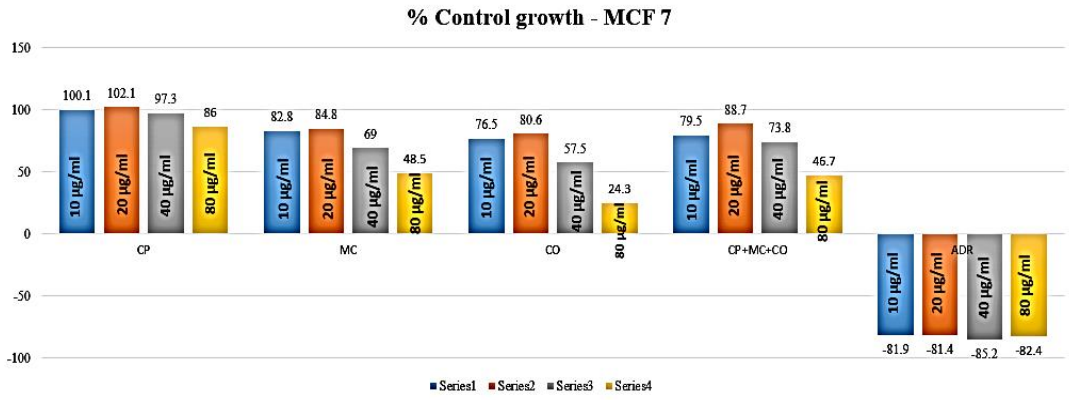

Figure 3: Extracts showing inhibition of percent control growth in a dose dependent manner – MCF 7.

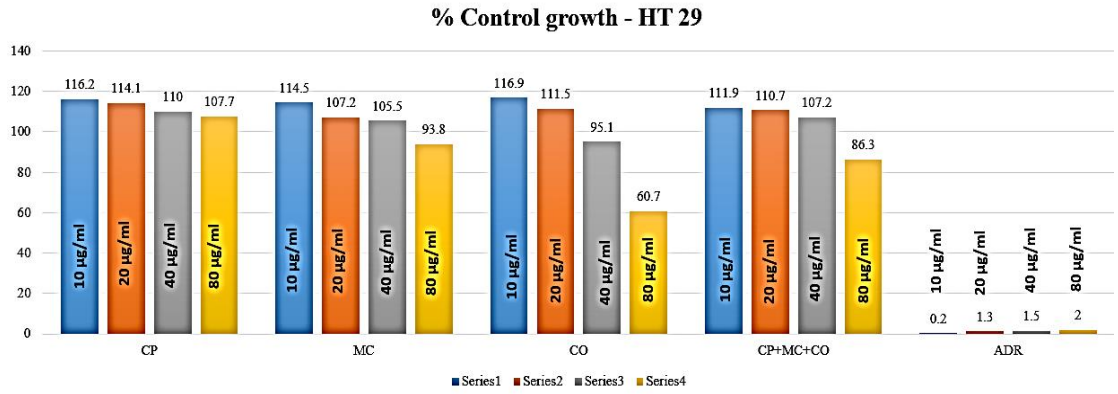

Figure 4: Extracts showing inhibition of percent control growth in a dose dependent manner – HT 29.

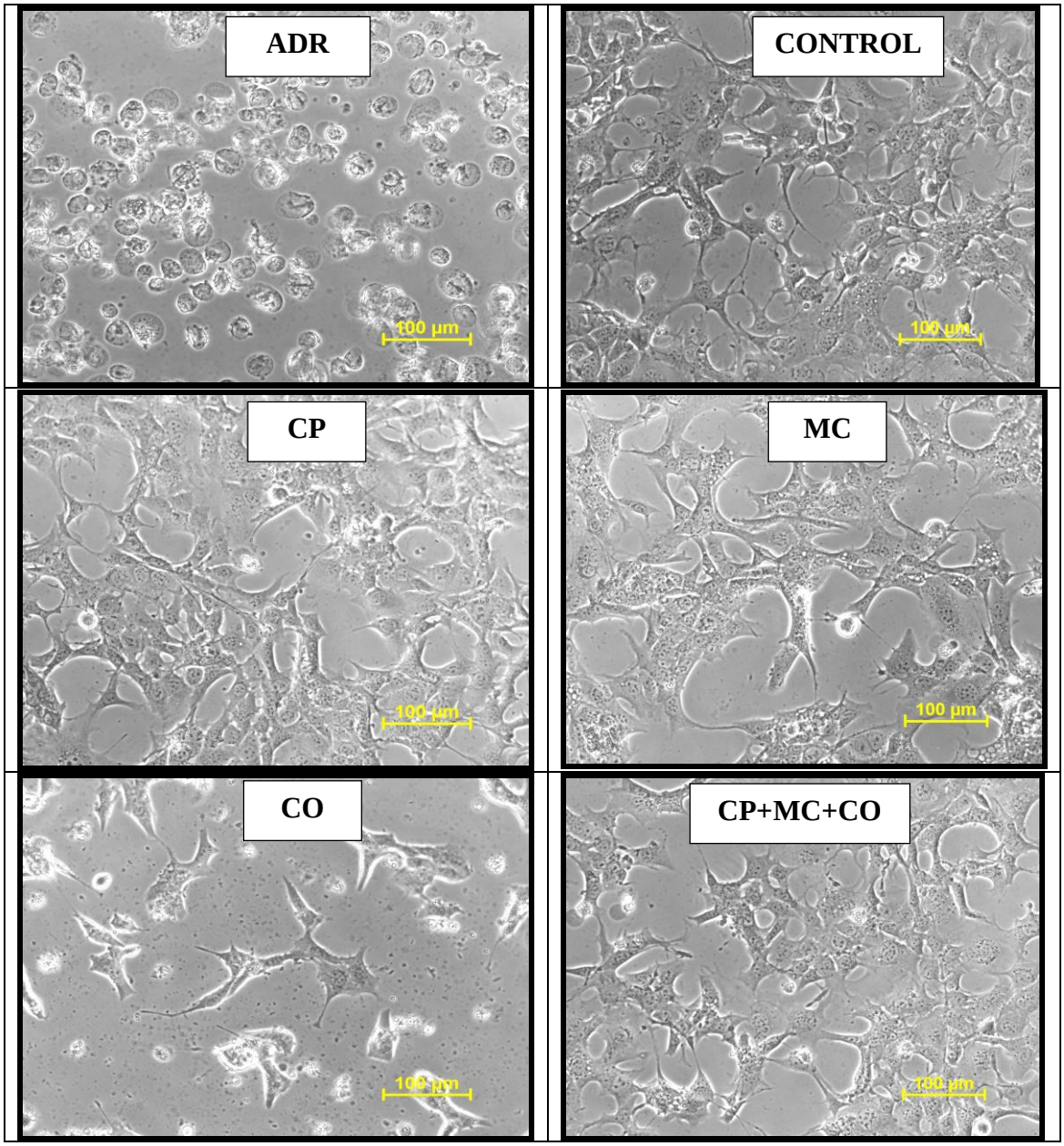

Figure 5: Morphology of MCF-7 when treated with standard adriamycin, control & ethanolic extracts of CP, MC, CO, and CP+MC+CO.

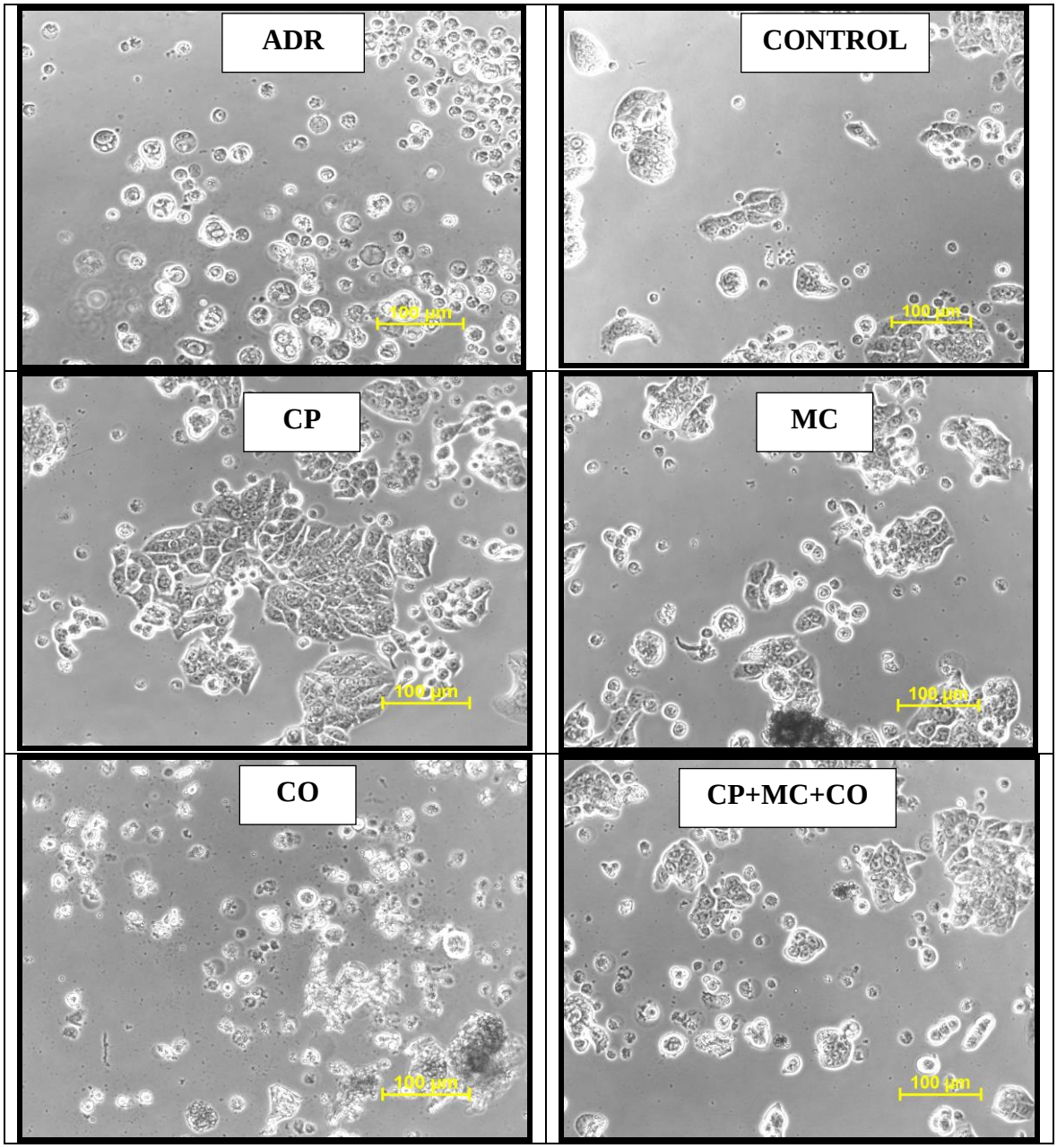

Figure 6: Morphology of HT-29 when treated with standard adriamycin, control & ethanolic extracts of CP, MC, CO, and CP+MC+CO.

CONCLUSION

All the extracts inhibited the percent control growth in dose dependent manner for both human breast and colon cancer cell line. GI50 concentration against both the cancer lines indicated unsatisfactory anticancer activity of extracts at the selected concentrations of 10, 20, 40 and 80 µg/ml.

ACKNOWLEDGEMENT

We would like to thank Dr. Jyoti Kode of Anti-cancer drug screening facility (ACDSF) at ACTREC, Tata Memorial Centre, Navi Mumbai for her valuable time and guidance relating to *in-vitro* activity. We would also like to acknowledge the management of Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan for their constant support in providing the required facilities for conducting this work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Comsa S, Maria AC, Raica M. The story of MCF-7 breast cancer cell line: 40 years of experience in research. Anticancer research, 2015; 35: 3147-3154.
2. Gheytanchi E, Naseri M, Busheri FK, Atyabi F, Mirsharif ES, Bozorgmehr M, Ghods R, Madjd Z. Morphological and molecular characteristics of spheroid formation in HT-29 and Caco-2 colorectal cancer lines. Cancer cell international, 2021; 21: 1-16.
3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. A cancer journal for clinicians, 2020; 70(1): 7-30.
4. Siegel RL, Miller KD, Wagle NS. Cancer statistics 2023. A cancer journal for clinicians, 2023; 73(1): 17-48.
5. Keller PJ, Lin AF, Arendt LM, Klebba I, Jones AD, Jenny A, Rudnick, DiMeo TA, Gilmore H, Jefferson DM, Graham RA, Naber SP, Schnitt S, Kuperwasser C. Mapping the cellular and molecular heterogeneity of normal and malignant breast tissues and cultured cell lines. Breast cancer research, 2010; 12: 1-17.
6. Maqueda DM, Miralles B, Recio I. HT29 cell line. The Impact of food bioactives on health: in vitro and ex vivo models, chapter 11, 2015.
7. Orellana EA, Kasinski AL. Sulforhodamine B (SRB) assay in cell culture to investigate cell proliferation. Bio-protocol, 2017; 6(21): 1-7.
8. Vanicha V, Kanyawim K. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nature Protocols, 2006; 1(3): 1112-1116.
9. Skehn P, Storeng R, Scudiero A, Monks J, Mc Mohan D, Vistica D, Jonathan T W, Bokesch H, Kenney S, Boyd. JNCI journal of the national cancer institute, 1990; 82: 1107-1112.
10. George BP, Chandran R, Abrahamse H. Role of phytochemicals in cancer chemoprevention: Insights. Antioxidants (Basel), 2021; 10(9): 1-33.
11. Kokate C.K., Practical Pharmacognosy, Delhi. India, Vallabh Prakashan, 1989; 111.
12. Gingine AP, Mandge SV, Jamkhande PG. Invitro evaluation of anticancer activity of methanolic extract of Annona reticulata linn. (ramphal) leaves on different human cancer cell lines. Journal of analytical and pharmaceutical research, 2016; 3(8): 1-3.
13. Yu H, Kim DJ, Choi HY, Kim SM, Rahaman MI, Kim YH, Kim SW. Prospective pharmacological methodology for establishing and evaluating anti-cancer drug resistant cell lines. BMC cancer, 2021; 21: 1-13.
14. Pawar M, Dwivedi J, Kataria U. Identification of phytochemicals in *Convolvulus pluricaulis*, *Michelia champaca* and *Chromolaena odorata* by orbitrap high resolution liquid chromatography mass spectroscopy (O-HRLCMS) and its pharmacological significance. International journal of biology, pharmacy and allied sciences, 2023; 12(10): 209-227.
15. Pawar MK, Dwivedi JA, Dwivedi UK. Structural elucidation of chemical compounds of pharmacological significance from convolvulus pluricaulis by gas chromatography: Mass spectroscopy (GC-MS) analysis. International journal of current advanced research, 2023; 12(7): 2221-2226.
16. Larsson P, Engqvist H, Biermann J, Ronnerman EW. Optimization of cell viability assays to improve replicability and reproducibility of cancer drug sensitivity screens. Scientific reports, 2020; 10(1): 1-12.