Journal of Applied and Natural Science
17(1), 407 - 420 (2025)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

In vitro assessment of antimitotic, antiproliferative and anticancer activities of different sections of *Cleistanthus collinus* (Roxb.) Benth. Ex. Hook. F.

S. Prathamanjali
Department of Botany, Sri Venkateswara University, Tirupati- 517502 (Andhra Pradesh), India
Y.T. Rajesh Babu*
Department of Botany, Andhra University, Visakhapatnam- 530003 (Andhra Pradesh), India
T. Vijaya
Department of Botany, Sri Venkateswara University, Tirupati- 517502 (Andhra Pradesh), India

Corresponding author. E-mail: baburajesh0999@gmail.com

Article Info
https://doi.org/10.31018/jans.v17i1.6324
Received: November 01, 2024
Revised: March 07, 2025
Accepted: March 13, 2025

How to Cite
Prathamanjali , S. *et al.* (2025). *In vitro* assessment of antimitotic, antiproliferative and anticancer activities of different sections of *Cleistanthus collinus* (Roxb.) Benth. Ex. Hook. F.. *Journal of Applied and Natural Science*, 17(1), 407 - 420. https://doi.org/10.31018/jans.v17i1.6324

Abstract
Natural-derived chemicals, particularly secondary metabolites from plants, are gaining attention for their potential as innovative and less harmful anticancer treatments, advancing cancer therapy and developing new medicinal herbs. The present study aimed to investigate the antimitotic, antiproliferative, and anticancer activities of various extracts from the leaves, bark, and fruits of *Cleistanthus collinus* using the *Allium cepa* root tip assay, yeast cell model, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay against Henrietta Lacks (HeLa) and Michigan Cancer Foundation-7 (MCF-7) cancer cell lines. For the MTT assay, crude methanol extract was used, while methanol, ethyl acetate, chloroform, aqueous, and petroleum ether extracts were employed for the antimitotic and antiproliferative assays. In the antimitotic assay, the methanol bark extract exhibited the highest activity, with a mitotic index of 15±0.57% at 500µg/mL, significantly reducing cell division compared to the control (97±1.23%). The methanol bark fraction showed the strongest activity in the antiproliferative assay, with only 26.41±0.57% viable cells at 500µg/mL. The extracts reduced cell viability and increased cytotoxicity in a dose-dependent manner. The anticancer activities (half-maximal inhibitory concentration, IC50 values) ranged from 186.43±2.7µg/mL to 885.69±6.77µg/mL against the MCF-7 cell line and from 228.5±3.7µg/mL to 550.85±4.67µg/mL against the HeLa cell line, with the highest activity observed for the crude methanol extract of bark against MCF-7 cells. These findings suggest that methanol extracts of *C. collinus* could be a promising source of plant-based anticancer agents with antimitotic, antiproliferative, and antioxidant activities.

Keywords: Chemotherapy, Cytotoxic activity, Henrietta Lacks (HeLa) cancer cell line, Michigan Cancer Foundation-7 (MCF-7) cancer cell line, Secondary metabolites

INTRODUCTION

The incidence of cancer-related deaths grew by 17% between 2005 and 2015, highlighting the critical need for more widespread research into the development of innovative anticancer drugs in addition to existing ones (Ohiagu *et al*., 2021). Cancer is the world's second leading cause of death, accounting for around 9.6 million deaths in 2018. Cancer is responsible for around one out of every six deaths globally. Cancer kills around 70% of persons in low- and middle-income countries (Nelson *et al*., 2020). It is estimated that by 2030, there will be 26 million new cancer diagnoses and 17 million cancer deaths (Solowey *et al*., 2014).

Cancer is a multi-stage process marked by abnormal cell growth that can invade and damage normal cells. Factors such as cigarette smoking, chemical exposure, dietary issues, and environmental influences contribute to cancer development. Traditional cancer treatments often harm healthy tissue, and there are concerns about tumors' resistance to current therapies. Consequently, there is a pressing need for more effective treatments. Plant extracts, rich in biologically active compounds like alkaloids, flavonoids, phenolics, and carotenoids, offer promising potential for cancer research and treatment development (Babu *et al*., 2024). Plant-based medicines for treating various ailments have been used since ancient times, such as in Ayur-

veda, Unani, and Siddha, and have been a component of human civilization (Pandey *et al*., 2020).

The plant *Cleistanthus collinus* is indigenous to India and Sri Lanka, is listed as endangered by the IUCN, and may be found in dry forests in southern and central India (Kazi and Gude, 2022). According to the researchers, Cleistanthin B, isolated from the poisonous plant *C. collinus* (Roxb), displayed strong anticancer properties in *invitro* studies. The anticancer activity of the complete plant's alcoholic extract is tested in human epidermoid carcinoma of the nasopharynx in culture, Walker carcinoma sarcoma 256 in rats, and L-1210 lymphoid leukemia in mice. The extract showed significant anticancer activity against human epidermoid nasopharyngeal carcinoma (Suman and Elangomathavan, 2013). In this study, the less investigated *C. collinus* plant extracts are examined for antimitotic, antiproliferative, and anticancer activities in HeLa and MCF-7 cells in an attempt to find a potential anticancer agent.

MATERIALS AND METHODS

Plant material collection and extraction of phytochemicals

Cleistanthus collinus leaves, bark, and fruits were collected from Gopavaramvillage (17˚22'09.16" N; 81˚47'48.42" E), Maredumilli forest zone of ASR District, Andhra Pradesh, India. The fresh leaves, fruits, and bark were thoroughly washed, shade-dried, mechanically pulverized, and stored. The phytochemicals were extracted using the soxhlet extraction method. 30 g of dry powder was extracted with 300 mL of solvents, comprising petroleum ether, chloroform, ethyl acetate, water, and methanol separately. The extracts were concentrated in a vacuum rotary evaporator at 60°C. The crude extracts obtained after thorough drying were utilized to evaluate potential antimitotic, antiproliferative, and anticancer effects.

Antimitotic activity assay

The antimitotic potency of *C. collinus* plant extracts was assessed using an *Allium cepa* root test using a slightly modified approach (Sehgal *et al*., 2006). *A. cepa* bulbs (40±15 g) were obtained from the Visakhapatnam local market and grown in water at room temperature until rooted. The bulbs with roots were immersed in dilutions of various plant extracts (100, 200, 300, 400, and 500 mg/mL) and incubated for 24 hours. Water was used to dilute the sample, and lapachol was used as the research standard. After 24 hours, the root tips were stabilized in a fixing solution, including acetic acid and alcohol (1:3). Acetocarmine was used to dye the squash mixture. The morphology and number of cells were evaluated under a microscope (40x). In all, 350-400 cells were counted, and cells in various stages of mitosis were recorded, including interphase (I) and prophase (P), metaphase (M), anaphase (A), and telophase (T). The mitotic index was calculated and compared to the control group using the following formula:

Mitotic index= [P + M + A + T/ Total cells] X 100 Eq. 1

Antiproliferative activity

The antiproliferative activity was assessed using a yeast cell model according to the approach of Saboo *et al*. (2008).

Yeast inoculum preparation

The yeast was inoculated with sterilized potato dextrose broth and incubated at 37°C for 24 hours, yielding seeded broth.

Determination of cell viability

A test tube combined 2.5 mL of potato dextrose broth (PDB) with 1 mL of each extract dilution and 0.5 mL of yeast inoculum. The control consisted of simply PDB and yeast inoculum. Quercetin was the standard antiproliferative medication. All test tubes were incubated for 24 hours at 37 degrees Celsius. After incubation, each sample's cell suspension was combined with 0.1% methylene blue and viewed using a low-power (10 ×) microscope. The number of viable cells, those that did not stain and seemed transparent with an oval shape, and dead cells, that stained and appeared blue, were counted in the Hemocytometer's 16 chambers, and the average number of cells per mL was computed. The percentages of cell viability and cytotoxicity were calculated using the formula below.

Cell viability (%) = Total viable cells/Total cells X 100 Eq. 2

Cytotoxicity (%) = No. of dead cells/ Total cells X 100 Eq.3

Anticancer activity

Cell culture, maintenance of cell lines, growth medium, and treatment conditions

MCF-7 (Breast adenocarcinoma) and HeLa (cervical carcinoma) were collected from American Type Culture Collection (ATCC), Bangalore, India. Cell lines were maintained in Dulbecco's modified eagle medium (DMEM) with FBS (10%), Foetal Calf Serum (FCS) (2–10%), penicillin (100 units/ml) and streptomycin (100 µg/ml) at 37˚C with 5% CO_2. Cell culture was performed using standard procedures in a laminar airflow chamber (Weiskirchen *et al*., 2023).

MCF-7 breast cancer cells were grown in minimal essential medium (MEM) alpha and human cervical carcinoma cells (HeLa). Cells were kept at 37°C in a 5% CO_2 humidified incubator. Cells were passaged at 80% confluency, with medium refreshed every 2-3 days (Maqsood *et al*., 2018).

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The cytotoxicity of *C. collinus* leaf, bark, and fruit methanol solvent extracts on the MCF-7 and HeLa cell lines was assessed using the MTT test (Van *et al*., 2011). The cells (10000 cells/well) were grown in 96-well plates for 24 hours in DMEM media with 10% FBS and 1% antibiotic solution at 37°C with 5% CO_2. Cells were treated the next day with formulations at concentrations ranging from 1-1000 µg/ml (made in an incomplete medium). After 24 hours of incubation, MTT Solution (250µg/ml) was added to the cell culture and incubated for 2 hours. After the experiment, the culture supernatant was collected, and the cell layer matrix was dissolved in 100 µl of Dimethyl Sulfoxide (DMSO). The results were read at 540 and 660 nm using an Elisa plate reader (iMark, Biorad, USA).

RESULTS

Antimitotic activity screening

Cleistanthus collinus antimitotic screening results indicated that distilled water, used as a control, had a mitotic index of 97%, with cells actively dividing at various stages of mitosis. Plant extract *C. collinus* leaf, bark, and fruit extracts (methanol, ethyl acetate, chloroform, aqueous, and petroleum ether) concentrations from 100µg/mL to 500µg/mL, caused a dose-dependent decrease in mitotic indices compared to the control (P ≤0.05) (Fig. 1). At the highest concentration of 500µg/mL, the methanol bark fraction had the highest antimitotic activity against *Allium cepa* root tip cells, with a mitotic index of just 15±0.57%. Petroleum ether fruit extract had the lowest mitotic activity (84±0.57%), followed by petroleum ether bark fractions (81±0.57%). At a concentration of 100µg/mL, the methanol leaf fraction showed the highest antimitotic effectiveness (78±1.23%), whereas the petroleum ether fruit and leaf extracts had the lowest activity (94±1.23%). The data show that the antimitotic potentiality ranking order at 500 µg/mL concentration is bark methanol extract (CBM) > fruit methanol extract (CFM) > leaf methanol extract (CLM) > bark chloroform extract (CBC) > fruit chloroform extract (CFC) > leaf chloroform extract (CLC) > bark ethyl acetate extract (CBE) > leaf ethyl acetate extract (CLE) > fruit ethyl acetate extract (CFE) > leaf petroleum ether extract (CLP) > bark acetone extract (CBA) > leaf acetone extract (CLA) = fruit acetone extract (CFA) > bark petroleum ether extract (CBP) > fruit petroleum ether extract (CFP) (Tables 1 to 5). These findings suggest that the methanol fraction might be a viable alternative for separating plant-based anticancer medicines. The mitotic index results were analyzed using a one-way ANOVA, and Tukey's range test revealed that all extract dosages significantly reduced the mitotic index when compared to the negative control.

Antiproliferative activity analysis

All extracts (methanol, ethyl acetate, chloroform, aque-

Table 1. Antimitotic activity of methanol extracts of *Cleistanthus collinus* leaf, bark, and fruit extracts

Plant part	Concentration (µg/mL)	No. of Dividing cells of Allium cepa root tips					No. of non-Dividing cells	Mitotic index (%)
		P	M	A	T	Total		
Leaf (CLM)	100	71	4	1	2	78	22	78±1.23^{c}
	200	65	3	-	3	71	29	71±0.57^{c}
	300	41	2	1	2	46	54	46±1.52^{f}
	400	32	2	1	1	36	64	36±0.57^{g}
	500	15	-	-	-	15	85	29±0.57^{g}
Bark (CBM)	100	75	4	2	-	81	19	81±2.23^{b}
	200	69	2	1	1	73	27	73±1.52^{c}
	300	50	1	2	2	55	45	55±0.57^{e}
	400	38	2	-	2	42	58	42±0.75^{f}
	500	26	1	2	-	29	71	15±0.57^{g}
Fruit (CFM)	100	78	3	1	2	84	16	84±2.12^{b}
	200	64	2	2	1	69	31	69±0.57^{d}
	300	40	-	3	1	44	56	44±0.75^{f}
	400	33	2	-	2	37	63	37±0.57^{g}
	500	19	-	-	-	19	81	19±0.57^{g}
Control		86	6	2	3	97	3	97 ± 1.23^{a}

P: prophase, M: metaphase, A: anaphase, T: telophase. Total number of cells = 100. At P ≤ 0.05, values with different alphabets (a-g) are statistically distinct from one another; CLM: Leaf methanol extract, CBM: Bark methanol extract, CFM: Fruit methanol extract.

Table 2. Antimitotic activity of ethyl acetate extracts of *Cleistanthus collinus* leaf, bark, and fruit extracts

Plant part	Concentration (µg/mL)	No. of Dividing cells of Allium cepa root tips					No. of non-Dividing cells	Mitotic index (%)
		P	M	A	T	Total		
Leaf (CLE)	100	86	3	-	2	91	9	91±1.5[a]
	200	79	2	1	3	85	15	85±0.75[b]
	300	75	-	2	-	77	23	77±1.25[c]
	400	73	1	-	-	74	26	74±0.57[c]
	500	70	-	-	-	70	30	70±1.25[c]
Bark (CBE)	100	86	2	-	2	90	10	90±1.5[a]
	200	80	1	-	1	82	18	82±0.57[b]
	300	72	1	1	1	75	25	75±0.57[c]
	400	71	-	-	-	71	29	71±1.25[c]
	500	69	-	-	-	69	31	69±0.57[d]
Fruit (CFE)	100	90	1	-	1	92	6	92±2[a]
	200	90	-	-	1	91	9	91±1[a]
	300	83	1	2	-	86	14	86±0.75[b]
	400	78	-	1	-	79	21	79±1[c]
	500	71	1	-	2	74	26	74±0.57[c]
Control		86	5	2	2	95	5	97 ± 1.23[a]

P: prophase, M: metaphase, A: anaphase, T: telophase. Total number of cells = 100. At P ≤ 0.05, values with different alphabets (a-g) are statistically distinct from one another. CLE: Leaf Ethyl acetate extract, CBE: Bark Ethyl acetate extract, CFE: Fruit Ethyl acetate extract

Table 3. Antimitotic activity of chloroform extracts of *Cleistanthus collinus* leaf, bark, and fruit extracts

Plant part	Concentration (µg/mL)	No. of dividing cells of Allium cepa root tips					No. of non-Dividing cells	Mitotic index (%)
		P	M	A	T	Total		
Leaf (CLC)	100	82	2	-	2	86	14	86±1.5[b]
	200	80	-	1	-	81	19	81±0.75[b]
	300	72	-	-	2	74	26	74±1[c]
	400	65	1	-	-	66	34	66±0.57[d]
	500	52	-	-	-	52	48	59±0.57[e]
Bark (CBC)	100	83	3	1	1	88	12	88±1[b]
	200	73	1	-	2	76	24	76±0.57[c]
	300	67	-	1	1	69	31	69±1.25[d]
	400	60	-	-	1	61	39	61±1.25[d]
	500	59	-	-	-	59	41	52±0.57[e]
Fruit (CFC)	100	84	2	1	3	90	10	90±1.5[a]
	200	80	-	1	-	81	19	81±2[b]
	300	71	1	1	1	74	26	74±0.75[c]
	400	62	-	2	-	64	36	64±1[d]
	500	57	1	-	-	58	42	58±0.57[e]
Control		86	5	2	2	95	5	97 ± 1.23[a]

P: prophase, M: metaphase, A: anaphase, T: telophase. Total number of cells = 100. At P ≤ 0.05, values with different alphabets (a-g) are statistically distinct from one another. CLC: Leaf chloroform extract, CBC: Bark chloroform extract, CFC: Fruit chloroform extract.

ous, and petroleum ether) derived from *C. collinus* leaf, bark, and fruit samples were evaluated for antiproliferative activity against yeast cells. Tables 6-10 exhibit the experimental results. Distilled water (control) has 92.40±2.36% yeast cell viability, whereas Quercetin (1mg/mL) had 61.06±1.23%.The percentages of actively proliferating cells and dead cells vary amongst *C. collinus* extracts. As plant extract concentrations grew from 100µg/mL to 500µg/mL, the number of dividing cells decreased, resulting in a dose-dependent reduction in mitotic indices compared to the control (P <0.05) (Fig. 2).

Methanol extracts had the highest antiproliferative effects. The methanol bark extract showed the highest

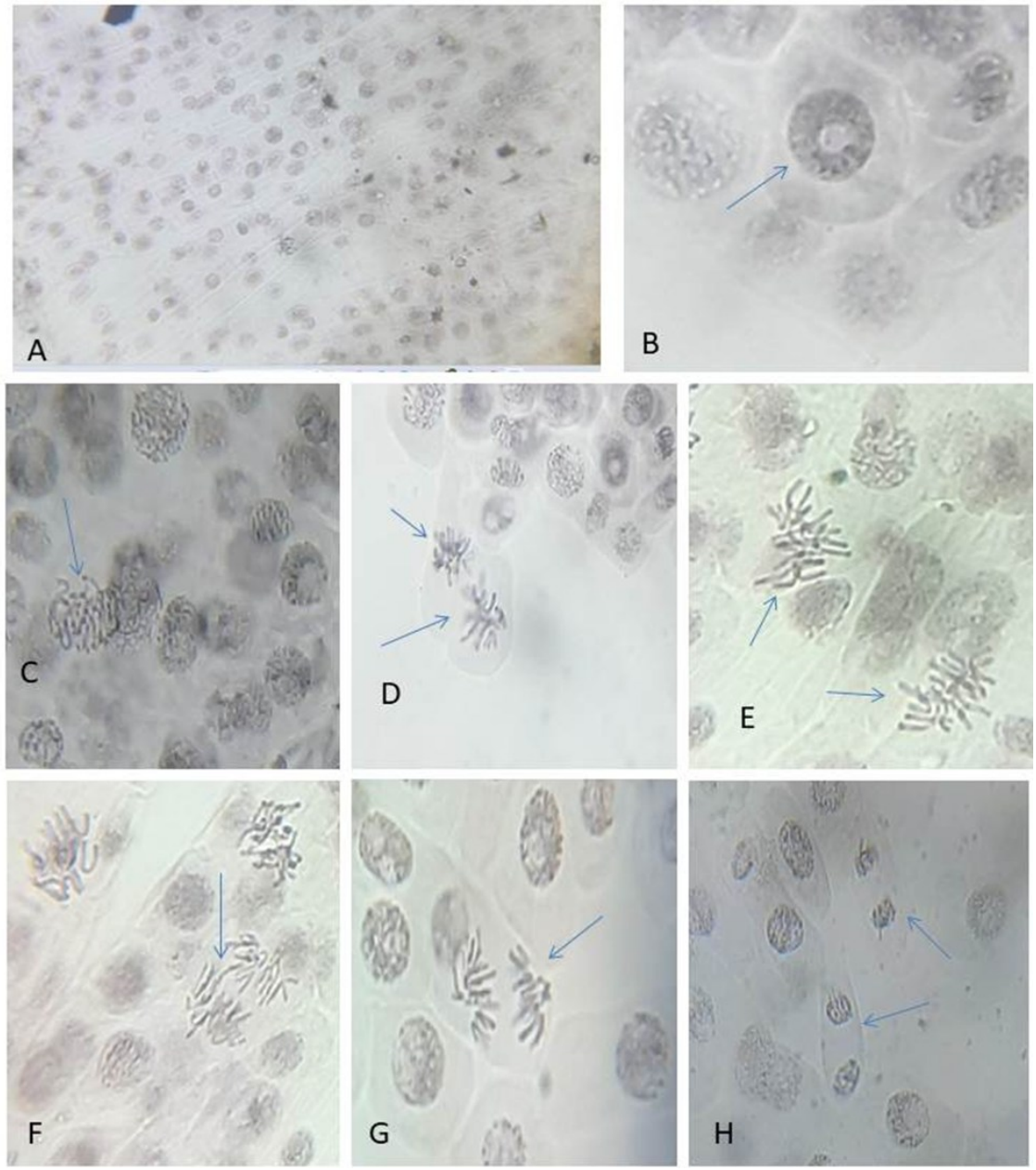

Fig. 1. *Photographs showing antimitotic work. A) Control, B) Interphase in A. cepa root tip cells treated with fruit methanol extract (500µg/mL), C) Prophase in A. cepa root tip cells treated with leaf methanol extract(300µg/mL), D & E) Metaphase in A. cepa root tip cells treated with fruit ethyl acetate extract (200µg/mL), F & G) Anaphase in A. cepa root tip cells treated with bark acetone extract (200µg/mL) and H) Telophase in A. cepa root tip cells treated with leaf chloroform extract(100µg/mL).*

activity at 500µg/mL, reducing yeast cell viability to 26.41±0.57%. The leaf extract reduced cell viability from 85.50% to 31.16%, and the bark extract from 86.25% to 26.41%. The fruit extract showed a decrease from 88.53% to 31.14%. Ethyl acetate extracts also showed significant antiproliferative effects. Leaf extract viability dropped from 83.50% to 54.32%, bark extract from 85.11% to 42.94%, and fruit extract from 86.55% to 58.43% with increasing concentrations.

Chloroform extracts exhibited considerable antiproliferative activity. Leaf extract viability decreased from 83.96% to 35.06%, bark extract from 85.76% to 32.03%, and fruit extract from 91.64% to 32.12%. Aqueous extracts demonstrated noteworthy antiproliferative potential. Leaf extract viability decreased from 86.98% to 50.27%, bark extract from 88.38% to 35.16%, and fruit extract from 90.54% to 54.34%. Petroleum ether extracts showed the lowest antiproliferative activities. Leaf extract viability dropped from 83.33% to 52.07%, bark extract from 85.98% to 41.26%, and fruit extract from 91.44% to 69.59%. Statistical tests (ANOVA and Tukey's range test) indicated a substantial decrease in yeast cell viability at all extract dosages compared to the negative control. This

Table 4. Antimitotic activity of aqueous extracts of *Cleistanthus collinus* leaf, bark, and fruit extracts

Plant part	Concentration (µg/mL)	No. of Dividing cells of Allium cepa root tips					No. of non-Dividing cells	Mitotic index (%)
		P	M	A	T	Total		
Leaf (CLA)	100	89	-	2	1	93	7	96±2[a]
	200	90	1	-	-	91	9	91±1.25[a]
	300	82	2	2	-	86	14	86±0.57[b]
	400	81	-	-	-	81	19	81±1.5[b]
	500	79	-	-	-	79	21	79±1[c]
Bark (CBA)	100	90	1	2	3	96	4	93±1[a]
	200	90	-	1	1	92	8	92±0.57[a]
	300	87	1	1	-	89	11	89±1[b]
	400	81	-	-	2	83	17	83±0.75[b]
	500	81	-	-	-	81	19	77±0.57[c]
Fruit (CFA)	100	92	-	1	-	92	3	93±1.23[a]
	200	90	-	-	1	91	9	91±1[a]
	300	84	1	-	1	86	14	86±0.57[b]
	400	79	-	-	-	79	21	81±0.57[b]
	500	76	1	-	-	77	23	79±1[c]
Control		86	5	2	2	95	5	97 ± 1.23[a]

P: prophase, M: metaphase, A: anaphase, T: telophase. Total number of cells = 100. At P ≤ 0.05, values with different alphabets (a-g) are statistically distinct from one another. CLA: Leaf aqueous extract, CBA: Bark aqueous extract, CFA: Fruit aqueous extract

Table 5. Antimitotic activity of petroleum ether extracts of *Cleistanthus collinus* leaf, bark, and fruit extracts

Plant part	Concentration (µg/mL)	No. of Dividing cells of Allium cepa root tips					No. of non-Dividing cells	Mitotic index (%)
		P	M	A	T	Total		
Leaf (CLP)	100	90	2	1	1	94	5	94±1.23[a]
	200	87	-	1	1	89	11	89±1.23[b]
	300	85	1	-	-	86	14	86±0.57[b]
	400	77	-	1	1	79	21	79±1[c]
	500	75	1	-	-	76	24	76±0.57[c]
Bark (CBP)	100	90	3	2	1	95	5	95±2[a]
	200	89	-	1	1	91	9	91±1.5[a]
	300	88	1	-	-	89	11	89±0.57[b]
	400	83	-	-	-	83	17	83±0.57[b]
	500	79	1	1	-	81	19	81±0.57[b]
Fruit (CFP)	100	90	-	3	1	94	2	94±1.23[a]
	200	90	1	-	-	91	9	91±0.75[a]
	300	85	2	-	1	88	12	88±0.57[b]
	400	85	-	-	1	86	14	86±1[b]
	500	84	-	-	-	84	16	84±0.57[b]
Control		86	5	2	2	95	5	97 ± 1.23[a]

P: prophase, M: metaphase, A: anaphase, T: telophase. Total number of cells = 100. At P ≤ 0.05, values with different alphabets (a-g) are statistically distinct from one another. CLP: Leaf petroleum ether extract, CBP: Bark petroleum ether extract, CFP: Fruit petroleum ether extract

study highlights the varied antiproliferative properties of *C. collinus* extracts, establishing a framework for further investigation in cancer research.

Anticancer activity human breast (MCF-7) cell line

The cell viability of *C. collinus* leaf, bark, and fruit methanol solvent extracts tested for cytotoxicity on the human breast (MCF-7) cell line at different concentrations (0, 1, 10, 50, 100, 250, 500, and 1000-µg/mL) exhibited dose-dependent effects on cell viability and cytotoxicity in cancer cell lines (Figures 3-4). The leaf extract showed variable cell viability, decreasing from 95.3% at 1 µg/mL to 34.3% at 1000 µg/mL, with corresponding increases in cytotoxicity from 4.7% to 65.8%. The bark extract demonstrated a stronger cytotoxic effect, with cell viability dropping from 81.1% at 1 µg/mL to 22.1% at 1000 µg/mL, and cytotoxicity rising from 18.9% to 77.9%. The fruit extract displayed the least potency at

Table 6. Antiproliferative activity of methanol extracts of *C. collinus* leaf, bark, and fruit extracts

Plant part	Concentration (µg/mL)	Average no. of total yeast cells	Average no. of live yeast cells	No. of non-dividing yeast cells	Yeast cell viability (%)
	100	400	342	58	85.50±1.52[a]
	200	383	238	145	62.14±0.57[c]
Leaf	300	358	184	174	51.40±1.23[d]
	400	391	148	243	37.85±0.57[e]
	500	398	124	274	31.16±0.57[e]
	100	371	320	51	86.25±2.23[a]
	200	378	225	153	59.52±1.52[c]
Bark	300	392	194	198	49.49±0.57[d]
	400	366	125	241	34.15±0.75[e]
	500	390	103	287	26.41±0.57[e]
	100	375	332	43	88.53±2.12[a]
	200	406	237	169	58.37±0.57[c]
Fruit	300	377	184	193	48.81±0.75[d]
	400	370	142	228	38.38±0.57[e]
	500	350	109	241	31.14±0.57[e]
Controls					
Distilled water		382	353	29	92.40±2.36[a]
Quercetin (1 mg/mL)		375	229	280	61.06±1.23[c]

* Mean values with different letters (a–e) are significantly different ($p<0.05$) based on Tukey's honestly significant difference comparisons

Table 7. Antiproliferative activity of ethyl acetate extracts of *C. collinus* leaf, bark, and fruit extracts

Plant part	Concentration (µg/mL)	Average no. of total yeast cells	Average no. of live yeast cells	No. of non-dividing yeast cells	Yeast cell viability (%)
	100	394	329	65	83.50±1.23[b]
	200	441	316	125	71.66±0.57[b]
Leaf	300	449	295	154	65.70±1.25[c]
	400	451	264	187	58.54±0.57[c]
	500	440	239	201	54.32±0.75[d]
	100	403	343	60	85.11±2.12[a]
	200	463	318	145	68.68±1.34[c]
Bark	300	461	274	187	59.44±0.75[c]
	400	478	232	246	48.54±0.57[d]
	500	510	219	291	42.94±1.52[d]
	100	357	309	48	86.55±2. 32[a]
	200	372	287	85	77.15±0.57[b]
Fruit	300	355	261	94	73.52±0.75[b]
	400	369	244	125	66.12±0.75[c]
	500	344	201	143	58.43±0.57[c]
Controls					
Distilled water		382	353	29	92.40±2.36[a]
Quercetin (1 mg/mL)		375	229	280	61.06±1.23[c]

* Mean values with different letters (a–d) are significantly different ($p<0.05$) based on Tukey's honestly significant difference comparisons

lower doses, with cell viability decreasing from 98.2% at 1 µg/mL to 48.3% at 1000 µg/mL, and cytotoxicity increasing from 1.8% to 51.7%.Comparing the extracts, the bark extract showed the highest cytotoxicity at lower concentrations, indicating its potent effect on reducing MCF-7 cell viability. The fruit extract was less effective at lower doses but still demonstrated significant cytotoxic effects at higher concentrations. The leaf extract exhibited intermediate cytotoxicity. These findings suggest that the methanolic extracts of *C. collinus*, particularly the bark, have strong potential as anticancer agents. Doxorubicin, used as a positive control, significantly reduced cell viability to less than 23% in HeLa cells and 37% in MCF-7 cells, highlighting the potent cytotoxic effects of these plant extracts in comparison.

The cytotoxic activity of methanol extracts from *C. collinus* leaves, bark, and fruit was evaluated against the MCF-7 breast cancer cell line, with cytotoxicity expressed as IC_{50} values, representing the concentration required to reduce cell viability by half (Table 11). The bark extract exhibited the highest cytotoxicity with an IC_{50} of 186.43±2.7 µg/mL, indicating its potent effect

Table 8: Antiproliferative activity of chloroform extracts of *C. collinus* leaf, bark, and fruit extracts

Plant part	Concentration (µg/mL)	Average no. of total yeast cells	Average no. of live yeast cells	No. of non-dividing yeast cells	Yeast cell viability (%)
	100	374	314	60	83.96±2.32^{b}
	200	386	294	92	76.16±0. 57^{c}
Leaf	300	394	210	184	53.30±1.25^{d}
	400	374	164	210	43.85±0.57^{d}
	500	405	142	263	35.06±0.57^{e}
	100	344	295	49	85.76±2.12^{a}
	200	392	264	128	67.35±1.32^{c}
Bark	300	365	218	147	59.73±0.47^{c}
	400	381	163	218	42.78±0.75^{d}
	500	434	139	295	32.03±0.35^{e}
	100	383	351	32	91.64±1.52^{a}
	200	387	284	103	73.39±0.75^{b}
Fruit	300	375	218	157	58.13±0.45^{c}
	400	361	146	215	40.44±0.57^{d}
	500	386	124	262	32.12±0.67^{e}
Controls					
Distilled water		382	353	29	92.40±2.36^{a}
Quercetin (1 mg/mL)		375	229	280	61.06±1.23^{c}

* Mean values with different letters (a–e) are significantly different ($p<0.05$) based on Tukey's honestly significant difference comparisons

Table 9: Antiproliferative activity of aqueous extracts of *C. collinus* leaf, bark, and fruit extracts

Plant part	Concentration (µg/mL)	Average no. of total yeast cells	Average no. of live yeast cells	No. of non-dividing yeast cells	Yeast cell viability (%)
	100	361	314	47	86.98±1.34^{a}
	200	370	286	84	77.30±0.65^{b}
Leaf	300	367	251	116	68.39±1.23^{c}
	400	367	214	153	58.31±0.52^{c}
	500	376	189	187	50.27±0.75^{d}
	100	370	327	43	88.38±2.12^{a}
	200	383	315	68	82.25±1.25^{b}
Bark	300	368	273	95	74.18±0.75^{b}
	400	384	231	153	60.16±0.68^{c}
	500	380	133	247	35.16±0.48^{e}
	100	370	335	35	90.54±2.32^{a}
	200	377	314	63	83.29±1.21^{b}
Fruit	300	381	285	96	74.80±1.12^{b}
	400	379	254	125	67.02±0.75^{c}
	500	368	200	168	54.34±0.57^{d}
Controls					
Distilled water		382	353	29	92.40±2.36^{a}
Quercetin (1 mg/mL)		375	229	280	61.06±1.23^{c}

* Mean values with different letters (a–e) are significantly different ($p<0.05$) based on Tukey's honestly significant difference comparisons

against MCF-7 cells. In comparison, the leaf extract had an IC_{50} of 531.52±4.96 µg/mL, suggesting moderate cytotoxicity, while the fruit extract showed the least cytotoxicity with an IC_{50} of 885.69±6.77 µg/mL. These findings highlight the potential therapeutic value of compounds in the bark extract for targeting breast cancer cells, as it demonstrated the most significant cytotoxic impact among the extracts tested.

Anticancer activity against Human cervical (HeLa) cancer cell line

The methanolic extracts of *C. collinus* (leaves, bark, and fruit) exhibited dose-dependent cytotoxicity against the HeLa cervical cancer cell line (Figures 5 and 6). For the leaf extract, cell viability significantly decreases while cytotoxicity rises with increasing concentrations. At 1 µg/mL, viability is reduced by 58.3%, and at 10µg/

Table 10. Antiproliferative activity of petroleum ether extracts of *C. collinus* leaf, bark, and fruit extracts

Plant part	Concentration (µg/mL)	Average no. of total yeast cells	Average no. of live yeast cells	No. of non-dividing yeast cells	Yeast cell viability (%)
	100	390	325	65	83.33±1.32[b]
	200	396	309	87	78.03±0.57[b]
Leaf	300	400	285	115	71.25±1.52[b]
	400	399	253	146	63.41±0.75[c]
	500	386	201	185	52.07±0.57[d]
	100	378	325	53	85.98±2.32[a]
	200	392	303	89	77.30±1.52[b]
Bark	300	399	284	115	71.18±0.75[b]
	400	380	243	137	63.95±0.57[c]
	500	378	156	222	41.26±0.48[d]
	100	374	342	32	91.44±2.45[a]
	200	375	326	49	86.93±1.21[a]
Fruit	300	377	301	76	79.84±0.75[b]
	400	393	296	97	75.32±0.57[b]
	500	374	264	110	69.59±0.45[c]
Control					
Distilled water		382	353	29	92.40±2.36[a]
Quercetin (1 mg/mL)		375	229	280	61.06±1.23[c]

* Mean values with different letters (a–d) are significantly different ($p<0.05$) based on Tukey's honestly significant difference comparisons

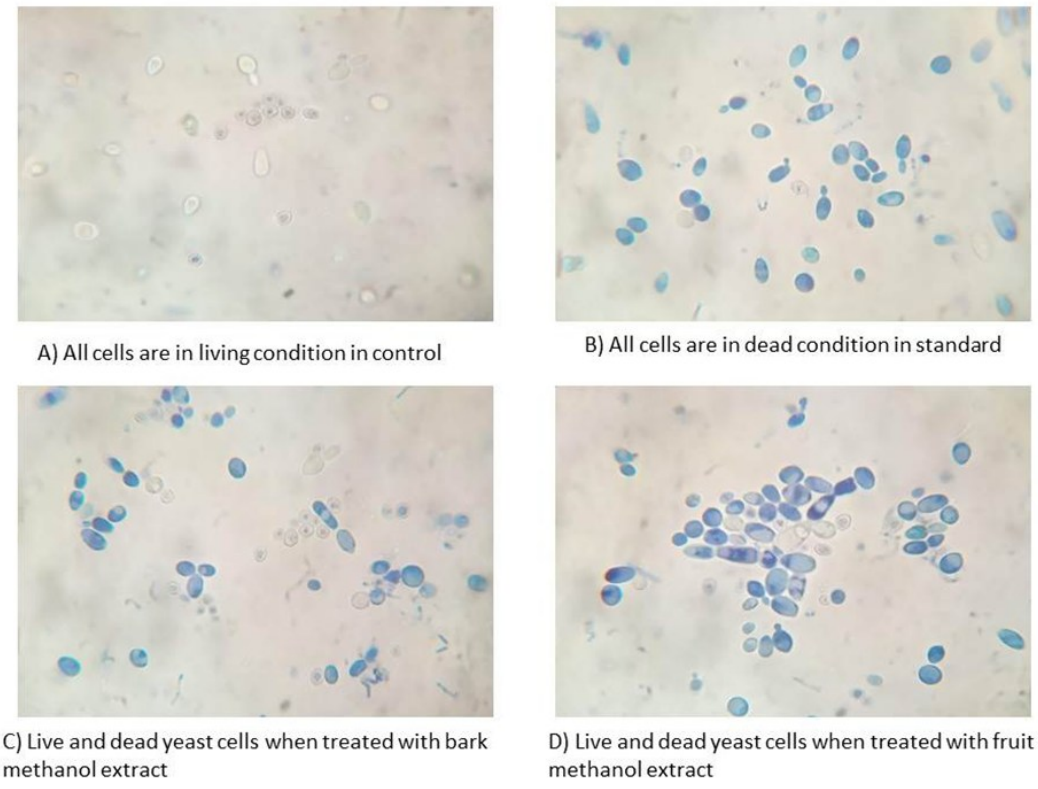

Fig. 2. *Microscopic images of Yeast cells in antiproliferative activity screening of various extracts*

mL, cytotoxicity reaches 48%. Higher doses, such as 50 and 100 µg/mL, show balanced harmful impacts, with viabilities dropping to 46% and corresponding increases in cytotoxicity. At 1000 µg/mL, viability plummets to 26.4%, with cytotoxicity peaking at 73.6%, indicating a strong cytotoxic effect at high doses.

Similarly, the methanolic bark extract shows substantial cytotoxicity, decreasing cell viability from 67.1% at 1 µg/mL to 21.3% at 1000 µg/mL, while cytotoxicity increases from 32.9% to 78.7%. The fruit extract follows a comparable pattern but starts with higher cell viability at lower doses, with 90.8% viability at 1 µg/mL. At 1000 µg/mL, the viability reduces to 32.3%, and cytotoxicity peaks at 67.7%. The results show a dose-dependent association between the concentration of fruit methanol extract and HeLa cell viability. As the concentration of

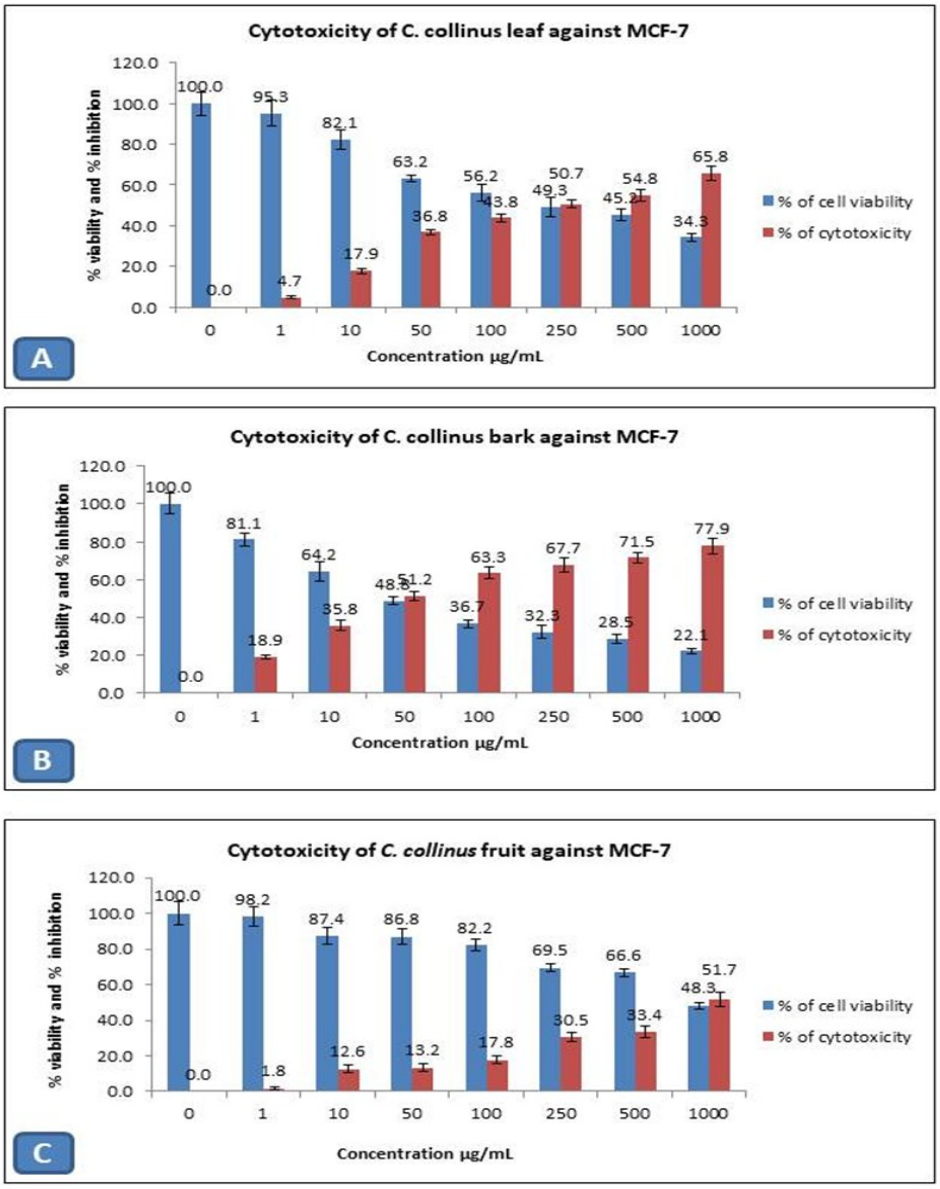

Fig. 3. *Methanol extract's cell viability and cytotoxicity percentage on breast cancer cell line MCF-7. A) Leaf methanol extract, B) Bark methanol extract, and C) Fruit methanol extract*

the extract increases, cell viability declines and cytotoxicity increases. These findings highlight the strong cytotoxic potential of the bark extract, followed by the leaf and fruit extracts, at greater doses against HeLa cells, suggesting their promise as anticancer agents.

The cytotoxic activity of different methanol extracts of *C. collinus* leaves, bark, and fruit was tested against the HeLa cervical cancer cell line, using IC_{50} values to determine the concentration required to inhibit 50% of cell viability (Table 12). Among the extracts, the bark methanol extract exhibited the greatest cytotoxic activity with an IC_{50} value of 228.5±3.7 µg/mL. The leaf extract had a higher IC_{50} value of 255.3±4.66 µg/mL, indicating moderate cytotoxicity, while the fruit extract showed the least cytotoxic action with the highest IC_{50} value of 550.85±4.67 µg/mL. These findings suggest that the methanol extract from the bark of *C. collinus* has the most significant cytotoxic impact on HeLa cervical cancer cells, followed by the leaf and fruit extracts. The potent cytotoxicity of the bark extract indicates potential therapeutic advantages for targeting cervical cancer cells.

DISCUSSION

The present study demonstrates the significant antimitotic, antiproliferative, and anticancer potential of *C collinus* extracts, particularly from the bark, against MCF-7 and HeLa cell lines. The methanol extract exhibited the highest cytotoxicity, with IC_{50} values of 186.43 ± 2.7 µg/mL for MCF-7 and 228.5 ± 3.7 µg/mL for HeLa cells (Tables 11 and 12), indicating its strong anticancer effi-

Table 11. Linear equations and IC_{50} Values of cytotoxicity of methanol extracts of *Cleistanthus collinus* on breast cancer cell line MCF-7

Plant species	Linear equation Y = mx + C	R^2 values	IC_{50}values[a] (µg/mL)
Bark extract	y = -0.0547x + 64.79	R^2 = 0.4839	186.43±2.7
Leaf extract	y = -0.0537x + 78.543	R^2 = 0.6126	531.52±4.96
Fruit extract	y = -0.0462x + 90.919	R^2 = 0.87	885.69 ±6.77

[a] Means of three determinations + SD (Standard deviation)

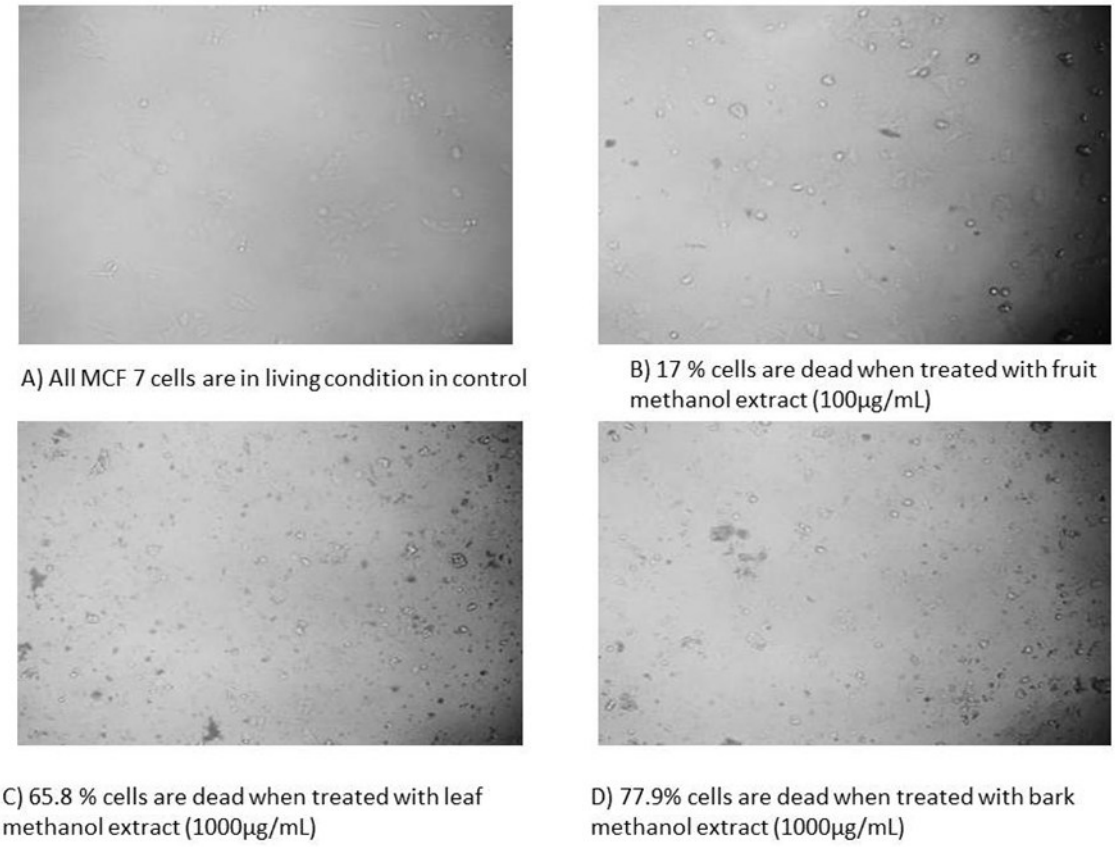

Fig. 4. *Microscopic images of MTT assay of MCF-7 cells treated with C. collinus methanol extracts*

cacy. Recent studies highlight the anticancer potential of *C. collinus*, primarily due to the presence of bioactive compounds such as cleistanthin A and B, which have been reported to induce apoptosis through DNA intercalation and topoisomerase inhibition (Jearawuttanakul *et al*., 2020; Sagar & Raveendran, 2022). Additionally, diterpenoids and flavonoids, commonly found in Euphorbiaceae, have been associated with cytotoxic effects against various cancer cell lines (Amtaghri *et al*., 2022). These compounds are likely contributors to the observed biological activity in the present study.

The dose-dependent cytotoxicity observed in MCF-7 and HeLa cells (Figures 4-6) indicates a broad-spectrum anticancer potential of *C.. collinus* extracts, with the bark extract exhibiting particularly potent effects. These findings align with previous studies demonstrating the cytotoxic efficacy of *C. collinus* extracts against nasopharyngeal epidermoid carcinoma and their selective toxicity towards proliferating cells (Priyadharsini *et al*., 2024). Furthermore, silver nanoparticles synthesized from *C. collinus* leaf extracts have been reported to enhance cytotoxic activity against osteosarcoma and lung cancer cells, reinforcing the therapeutic significance of this plant in cancer treatment (Kanipandian & Ramesh, 2024; Yugal *et al*., 2018). These results collectively highlight the potential of *C. collinus* as a promising candidate for further anticancer investigations, particularly in the context of targeted cancer therapies.

Furthermore, the antimitotic effects observed in the *Allium cepa* assay (Tables 1-5) support previous findings that phenolic-rich plant extracts interfere with microtubule formation, a characteristic seen in various

Table 12. Linear equations and IC_{50} Values of cytotoxicity of methanol extracts of *Cleistanthuscollinus*on cervical cancer cell line HeLa

Plant species	Linear equation Y = mx + C	R^2 values	IC_{50}values[a] (µg/mL)
Bark extract	y = -0.0481x + 60.991	R^2 = 0.472	228.5±3.7
Leaf extract	y = -0.0416x + 60.796	R^2 = 0.4301	255.33±4.66
Fruit extract	y = -0.0494x + 77.212	R^2 = 0.649	550.85±4.67

[a] Means of three determinations + SD (Standard deviation)

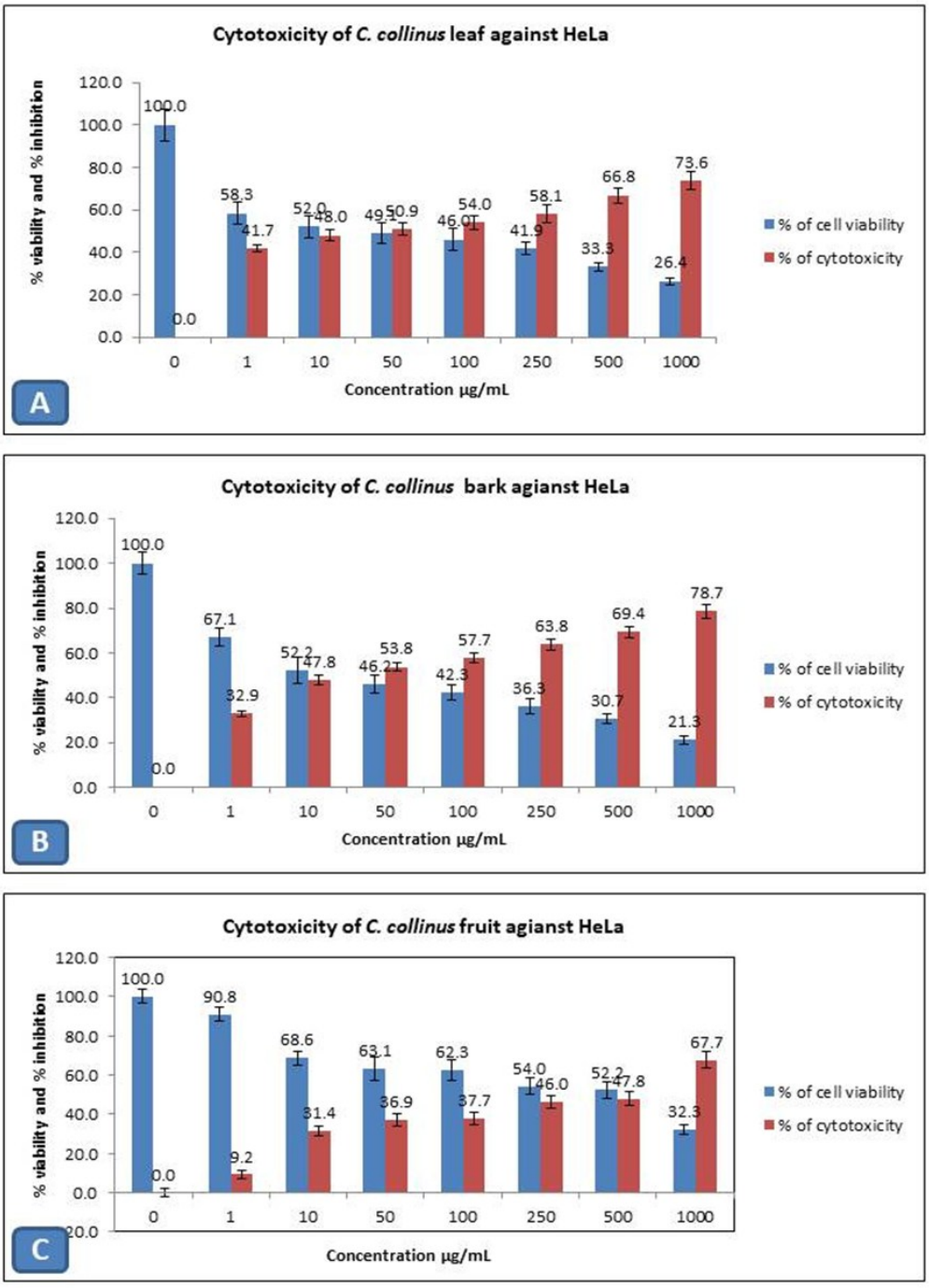

Fig. 5. *Methanol extract's cell viability and cytotoxicity percentage on breast cancer cell line HeLa. A) Leaf methanol extract, B) Bark methanol extract, and C) Fruit methanol extract*

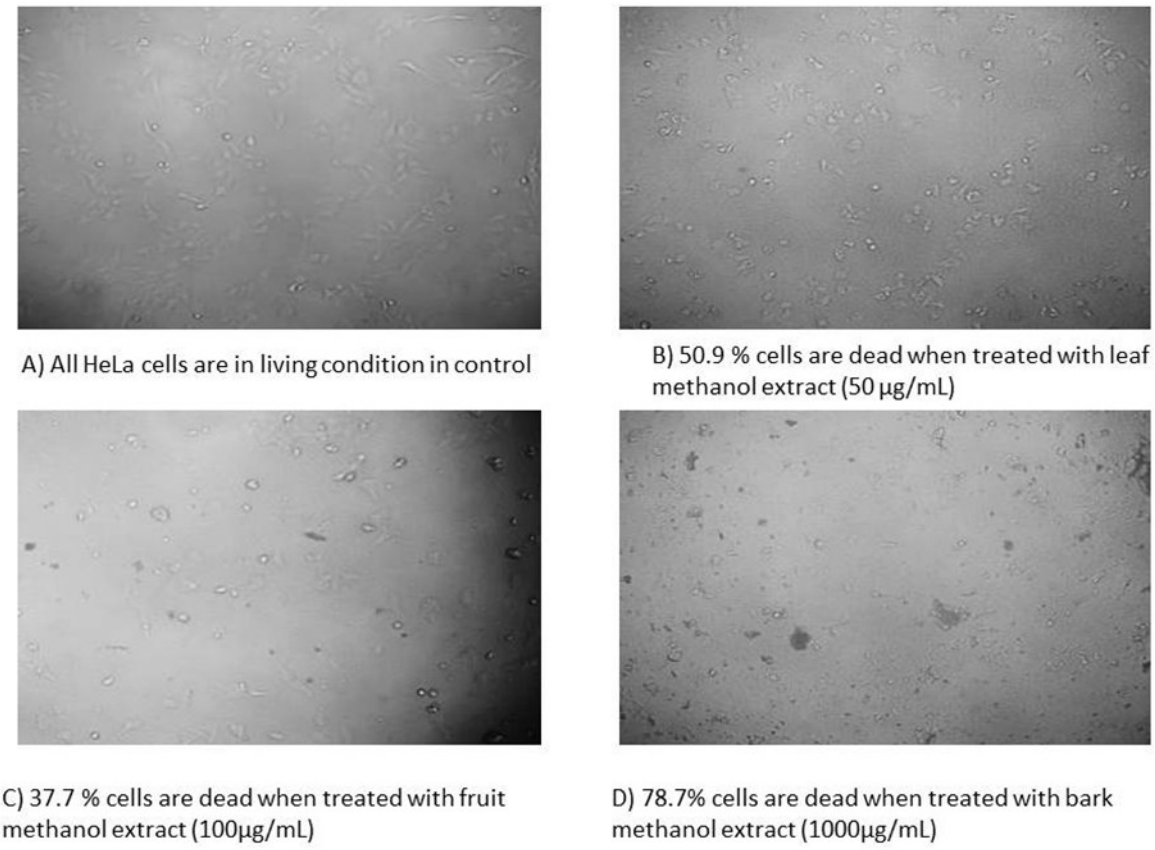

Fig. 6. *Microscopic images of MTT assay of HeLa cells treated with C. collinus methanol extracts*

flavonoid-containing Euphorbiaceae species (Magozwi *et al*., 2021). The antiproliferative effects observed in the yeast cell model (Tables 6-10) further reinforce the cytotoxic potential of *C. collinus*. The bark methanol extract showed the highest inhibition of yeast cell viability (26.41 ± 0.57% at 500µg/mL). Despite the growing interest in the pharmacological potential of *Cleistanthus* species, there remains a significant gap in studies evaluating their antimitotic and antiproliferative effects, particularly using the *Allium cepa* assay and yeast cell model. To the best of our knowledge, this study is the first to report the antimitotic and antiproliferative activities of *C. collinus*, providing new insights into its potential cytotoxic properties. While specific studies on the antiproliferative activities of *Cleistanthus* species in yeast models remain limited, research on related genera within Euphorbiaceae has demonstrated similar cytotoxic trends (Amtaghri *et al*., 2022).

Conclusion

Different extracts of *Cleistanthus collinus*, including methanol and ethyl acetate, exhibited varying levels of antimitotic and antiproliferative activities, with methanol extracts showing the strongest effects. The methanol bark extract was particularly potent, displaying significant cytotoxicity against MCF-7 and HeLa cancer cell lines, as indicated by lower IC_{50} values than leaf and fruit extracts. These findings highlight the potential of *C. collinus* bark as a source of biologically active compounds with anticancer properties, suggesting further research into *in vivo* studies and animal models to validate its therapeutic potential.

ACKNOWLEDGEMENTS

The authors are thankful to the Department of Botany, Sri Venkateswara University, for providing facilities and instruments for the research. The first author is thankful to Dr. Ummidi Ravi Shankar, Mic Gene Laboratory, Visakhapatnam, for his assistance during various stages of this research.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Amtaghri, S., Akdad, M., Slaoui, M., & Eddouks, M. (2022). Traditional uses, pharmacological, and phytochemical studies of *Euphorbia*: A review. *Current Pharmaceutical Design*, 22(19), 1553-1570. https://doi.org/10.2174/1568026622666220713143436
2. Babu, Y. T. R., Prathamanjali, S., Raju, B. A. R., Ramalakshmana, J., & Padal, S.B. (2024). Medicinal Plants from India as Prospects of Anticancer Drug Sources: a

Comprehensive Review. *In Futuristic Trends in Agriculture Engineering & Food Sciences*, 4 (3): 170–192. IIP Series. https://doi.org/10.58532/v3bcag4p2ch5

3. Jearawuttanakul, K., Khumkhrong, P., Suksen, K., Reabroi, S., Munyoo, B., Tuchinda, P., Borwornpinyo, S., Boonmuen, N., & Chairoungdua, A. (2020). Cleistanthin A induces apoptosis and suppresses motility of colorectal cancer cells. *European Journal of Pharmacology*, *889*, 173604. https://doi.org/10.1016/j.ejphar.2020.173604
4. Kanipandian, N., & Ramesh, R. (2024). Biosynthesis and Characterization of Silver Nanoparticles Using Cleistanthus collinus; Investigation on Molecular Mechanism of Apoptosis Cell Death Against Lung Cancer Cells. *Asian Pacific Journal of Cancer Biology*, *9*(4), 527–535. https://doi.org/10.31557/apjcb.2024.9.4.527-535
5. Kazi, M., and Gude, A. (2022). A review on poisonous, pesticidal and medicinal attributes of *Cleistanthuscollinus* (roxb.) benth. ex hook.f., *World Journal Of Pharmaceutical and Medical Research*, 8(4), 66 – 78.
6. Magozwi, D. K., Dinala, M., Mokwana, N., Siwe-Noundou, X., Krause, R. W. M., Sonopo, M., McGaw, L. J., Augustyn, W. A., & Tembu, V. J. (2021). Flavonoids from the Genus Euphorbia: Isolation, Structure, Pharmacological Activities and Structure–Activity Relationships. *Pharmaceuticals*, *14*(5), 428. https://doi.org/10.3390/ph14050428Nelson, V. K., Sahoo, N. K., Sahu, M., Sudhan, H. H., Pullaiah, C. P., & Muralikrishna, K. S. (2020). In vitro anticancer activity of *Eclipta alba* whole plant extract on colon cancer cell HCT-116. *BMC Complementary Medicine and Therapies*, 20(1). https://doi.org/10.1186/s12906-020-03118-9
7. Ohiagu, F. O., Chikezie, P. C., Chikezie, C. M., &Enyoh, C. E. (2021). Anticancer activity of Nigerian medicinal plants: a review. *Future Journal of Pharmaceutical Sciences*, 7(1). https://doi.org/10.1186/s43094-021-00222-6
8. Pandey, B. P., Adhikari, K., Pradhan, S. P., Shin, H. J., Lee, E. K., & Jung, H. J. (2020). In-vitro antioxidant, anti-cancer, and anti-inflammatory activities of selected medicinal plants from western Nepal. *Future Journal of Pharmaceutical Sciences*, 6(1). https://doi.org/10.1186/s43094-020-00107-0
9. Priyadharsini, R. P., Parasuraman, S., Puli, S., & Raveendran, R. (2024). A review on the poisonous plant *Cleistanthus collinus*. *Journal of Pharmacology and Pharmacotherapeutics*. https://doi.org/10.1177/0976500x241257741.
10. Saboo, S., Deore, S. L., Khadabadi, S. S., &Deokate, U. A. (2008). Evaluation of antimitotic and anticancer activity of the crude extracts of *Pterospermumacerifolium* wild leaves (sterculeaceae). *Nigerian Journal of Natural Products and Medicine*, 11 (1), 76–79. https://doi.org/10.4314/njnpm.v11i1.11888
11. Sehgal, R., Roy, S., & Kumar, V. L. (2006). Evaluation of the cytotoxic potential of latex of *Calotropis procera* and podophyllotoxin in *Allium cepa* root model. *Biocell: official journal of the SociedadesLatinoamericanas de Microscopia Electronica*, 30(1), 9–13.
12. Solowey, E., Lichtenstein, M., Sallon, S., Paavilainen, H., Solowey, E., &Lorberboum-Galski, H. (2014). Evaluating medicinal plants for anticancer activity. *The Scientific World Journal*, *2014*, 1–12. https://doi.org/10.1155/2014/721402
13. Suman, T., and Elangomathavan, R. (2013). Bioprospecting of *Cleistanthuscollinus* and its antibacterial activity. *Asian Journal of Pharmaceutical and Clinical Research,* 6(4):206-209.
14. Weiskirchen, S., Schroder, S. K., Buhl, E. M., & Weiskirchen, R. (2023). A beginner's guide to cell culture: Practical advice for preventing needless problems. *Cells*, 12(5), 682. https://doi.org/10.3390/cells12050682
15. Yugal, M. S., Kushwaha, P., & Pandey, B. (2018). Phytochemical Screening and Antioxidant Activity of Different Extracts of *Cleistanthuscollinus*. *Journal of Pharmacognosy and Phytochemistry*, 7(2), 123-127.