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Research Article

In vitro antioxidant and anticancer activity of phytochemical rich *Dendrobium bicameratum* Lindl. (Orchidaceae) leaf extracts against the MDA-MB-231 breast cancer cells

Amzad Basha Kolar[1]*, M. Rahamtulla[1], B. Sandhya[2], Diptendu Sarkar[3]

[1]P.G. Department of Botany, The New College (Autonomous), Affiliated to University of Madras, Chennai-600014, Tamil Nadu, India
[2]Department of Biotechnology, SIMS College of Life Sciences, Mangaldas Nagar, Guntur-522001, Andhra Pradesh, India
[3]Department of Microbiology, Ramakrishna Mission Vidyamandira (An Autonomous U.G. and P.G. College, Affiliated to University of Calcutta), Belur Math, Howrah-711202, West Bengal, India

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*Corresponding author: Amzad Basha Kolar, E-mail: amzadphd@gmail.com

Abstract

Dendrobium bicameratum is a promising epiphytic orchid used by tribal healers of the Eastern Ghats to treat various ailments. However, the plant has not been studied for bioactive properties. The present study investigates the phytochemical composition, antioxidant and anticancer properties of *D. bicameratum* leaf extracts on MDA-MB-231 breast cancer cells. Fresh leaf material of the plant was cold-extracted using various solvents. Phytochemical screening has been performed using standard qualitative and quantitative tests to detect the presence of phytochemicals. Antioxidant potential of the leaf extracts was determined using DPPH and FRAP assays. The anticancer property of various concentrations of the leaf extract on MDA-MB-231 breast cancer cells has been evaluated using an MTT assay. Phytochemical profiling results showed that the *D. bicameratum* methanol leaf extract indicated the presence of all the tested phytochemicals. However, the flavonoids and tannins are present in higher amounts. The same leaf extract displayed good antioxidant activity in both DPPH (IC_{50}=17.46±0.60 µg/mL) and FRAP (EC_{50}=101.12±0.94 µg/mL) assays. Furthermore, in the MTT assay, the above-mentioned extract reduced the viability of MDA-MB-231 cells in a dose-dependent manner (IC_{50}=3.84±0.62 µg/mL). Pearson correlation analysis showed that both antioxidant and anticancer activities were strongly concentration-dependent, with methanol and ethyl acetate leaf extracts showing high positive correlation coefficients (r=0.95–0.99) and significant p-values ($p<0.05$). This confirms that increasing leaf extract concentration consistently enhanced their free-radical scavenging, reducing power, and cytotoxic effects. Therefore, leaf extracts of *D. bicameratum* have notable antioxidant and anticancer potential, suggesting that they may serve as a reserve of bioactive metabolites of natural origin for breast cancer treatment.

Keywords: Orchidaceae, *Dendrobium bicameratum*, Phytochemicals, Antioxidant, Anticancer

Introduction

Orchidaceae is one of the important families in angiosperms, comprising 28,000 species across 763 genera (Christenhusz & Byng, 2016). Numerous orchid species with cultural significance have been used in herbal medicines and as food supplements by tribal people worldwide (Katta *et al.,* 2019). The genus *Dendrobium* comprises more than 1,600 species spread throughout tropical and subtropical Asia (Burzacka-Hinz *et al.,* 2025). Many *Dendrobium* species have been utilised to treat various ailments in traditional medicine (Cakova *et al.,* 2017). *Dendrobium bicameratum* is an epiphytic plant distributed in the forest areas of the Eastern Ghats. Traditionally, the leaf paste of *D. bicameratum* has been used by folk healers to treat skin-related ailments and breast lumps. However, the research work on the bioactivity of this plant is limited. The potential of this species as a source of antioxidant and anticancer compounds remains largely unexplored.

Cancer is a dreaded disease, and the very word scares most people. Breast cancer is the most predominant malignancy among women (Łukasiewicz *et al.,* 2021). Despite advancements in chemotherapeutic and targeted treatment strategies, the search for safer and more effective anticancer agents continues, as many existing medications cause severe side effects and resistance in tumour cells. Natural products obtained from medicinal plants have gained increasing attention because of their structural diversity, biocompatibility, and broad spectrum of biological activities, including antioxidant and anticancer effects (Izzo & Stefanska, 2025).

Ho and Chen (2003) studied the efficacy of moscatilin, extracted from *D. loddigesii*, and concluded it as an anticancer agent. Tsai *et al*. (2010) reported that moscatilin repressed the growth of the lung cancer cell line A549. Joshi *et al.* (2020) investigated the anticancer potential of *D. transparens* methanol extracts on HeLa and U251 cell lines. Paudel *et al.* (2022) stressed the importance of the genus *Dendrobium* in the treatment of cancer. Studies by Rahamtulla *et al.* (2023) revealed that the leaf extracts of *D. aphyllum* showed anticancer activity in the various human cancer cell lines. Therefore, screening plant extracts helps in identifying their potential therapeutic value against cancer.

Materials and methods

Collection and identification of Dendrobium bicameratum Lindl

A field survey was conducted in mid-June 2025 in the tribal settlements of the Paderu forest (Andhra Pradesh,

Eastern Ghats, India) for ethnobotanical investigation. During this field visit, *Dendrobium bicameratum* was identified in its natural habitats, and valuable ethnomedicinal data were procured through direct interactions with tribal healers. The plant was authenticated by consulting relevant taxonomic literature (Deva & Naithani, 1986; Prasad *et al.,* 2019) and a specimen (TNCBH1002) was deposited in the New College Botany herbarium (Chennai, Tamil Nadu) for future reference.

Preparation of leaf extract

The leaf samples of *D. bicameratum* are rinsed carefully with water, made into tiny pieces, dried at 26-28 °C for one month, and ground into a fine powder using an electric blender. Fifty grams of leaf powder was placed separately into 250 mL of solvents such as n-hexane, petroleum ether, ethyl acetate extract and methanol and cold extracted (Noorjahan *et al.,* 2024).

Phytochemical screening

Qualitative Phytochemical profiling of *D. bicameratum* leaf extracts has been performed using standard procedures (Harborne, 1973; Trease & Evans, 1989, 1996; Sofowara, 1993). The occurrence of major phytochemical groups such as alkaloids, flavonoids, phenols, saponins, steroids, tannins and terpenoids was determined based on characteristic colour changes or precipitate formation during chemical tests. The intensity of each phytochemical reaction has been recorded by means of a grading system, where '+++' indicates strong presence, '++' moderate, '+' slight presence, and '—' absence of the respective phytochemicals.

Quantitative estimation of secondary metabolites

The estimation of alkaloids was done as per the method of Mir *et al.* (2016), where the dried leaf powder was extracted, the alkaloids were precipitated, dried, and weighed to obtain the final percentage of alkaloids (dry weight basis). Total phenolics are measured by the Folin-Ciocalteau method (Baba & Malik, 2015), in which the leaf extract was reacted with appropriate reagents, and the absorbance is quantified at 765 nm to compute phenolic content as gallic acid equivalents (µg/mg of extract). Total flavonoids were quantified according to the Aluminium chloride assay of Liu *et al.* (2007), where the leaf extract was processed through the colorimetric reaction and the absorbance at 510 nm was used to express the flavonoid content in quercetin equivalents (µg/mg of extract).

Antioxidant assay

The antioxidant property of *D. bicameratum* leaf extracts was studied using the DPPH free radical scavenging (Blois, 1958) and the FRAP assays (Shabana *et al.,* 2024). Ascorbic acid is taken as the positive control, and antioxidant activity is calculated using the standard formulae given below.

$$\text{Percentage of DPPH radical inhibition} = \frac{\text{Ab} - \text{As}}{\text{Ab}} \times 100$$

$$\%\ \text{of}\ Fe^{3+}\ \text{reduction} = \frac{\text{As} - \text{Ab}}{\text{As}} \times 100$$

Ab=absorbance of control and As=absorbance of sample.

The IC_{50} value for DPPH and the EC_{50} value for the FRAP assay were estimated using Microsoft Excel based on the dose–response relationship. The percentage antioxidant activity was plotted against the logarithmic concentration of the *D. bicameratum* leaf extracts. A best-fit regression equation (linear trendline) is generated, and the IC_{50} and EC50 values have been evaluated. Mean values from triplicate experiments were used for analyses.

In vitro anticancer activity

In this study, the MTT assay has been used to study the anticancer property of *D. bicameratum* leaf extracts against the breast cancer cells (MDA-MB-231). The assay is carried out according to the protocol of Mosmann (1983). Briefly, the cultured cancer cells are seeded in 96-well plates at a density of 5×10^3 cells/well in 200 µL complete RPMI medium [RPMI + 10% fetal bovine serum (100 U/mL) + streptomycin (100 µg/mL) + gentamicin (50 µg/mL) + amphotericin B (0.50 µg/mL)] and incubated for 24 h. The medium is then replaced with fresh RPMI having various concentrations (1.56-100 µg/mL) of *D. bicameratum* leaf extracts. After 48 h of treatment, 10 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 h at 37 °C. The resulting formazan crystals were dissolved in DMSO for 1 h at room temperature, and plates were gently agitated until complete solubilization. Absorbance was noted at 595 nm with a microplate spectrophotometer.

Percentage cell death and cell viability are calculated using the formulae:

$$\text{Percentage of cell death} = \frac{\text{Ab} - \text{As}}{\text{Ab}} \times 100$$

Where Ab=absorbance of control and As=absorbance of sample

Percentage Viability = 100 - % Cell Death

The IC_{50} values were computed using Microsoft Excel. Percentage cell death was plotted against the logarithm of the *D. bicameratum* leaf extract concentrations, and regression analysis was executed to generate the best-fit trendline. The IC_{50} was calculated from the corresponding

linear regression equation (y=mx+c) by interpolating the concentration that produced 50% growth inhibition.

Results and discussion

Phytochemical profile of D. bicameratum leaf extracts

Phytochemical profiling of leaf extracts showed the existence of various phytochemicals in *D. bicameratum* (Table 1). All the tested phytocompounds were found in the methanol leaf extract. This extract also exhibited a strong presence of flavonoids and tannins, whereas other metabolites were present in moderate amounts. The presence of alkaloids, flavonoids, saponins and terpenoids is also recorded in the ethyl acetate leaf extract. These phytochemicals are recognised for displaying various pharmacological activities (Gantait *et al.,* 2021). Particularly, flavonoids and phenolic compounds exhibit effective antioxidant and anticancer properties. These secondary metabolites can scavenge reactive oxygen species (ROS) and hinder lipid peroxidation (Chandimali *et al.,* 2025). The presence of these phytochemicals in *D. bicameratum* suggests that the leaf extracts are a valuable source of antioxidant and anticancer compounds.

The results of the present study authenticate that *D. bicameratum* is rich in various secondary metabolites, supporting earlier reports on the genus *Dendrobium* (Wang, 2021). Sukumaran and Yadav (2016) carried out phytochemical profiling in *D. macrostachyum* and observed the presence of various phytochemicals, concluding that secondary metabolism in this plant appears to be a source of many biologically active metabolites. Previous research reports have also documented several phytochemicals in various *Dendrobium* species and their plant parts (Favre-Godal *et al.,* 2022; Noorjahan *et al.,* 2023; Fan *et al.,* 2025). In this study, n-hexane and petroleum ether leaf extracts of *D. bicameratum* exhibited poor phytochemical profiles, which may be credited to the limited solubility of many secondary metabolites (Tiwari *et al.,* 2011).

Table 1: Phytochemical composition of *Dendrobium bicameratum* leaf extracts

Phytochemicals	*Dendrobium bicameratum* Leaf			
	n-Hexane	Petroleum ether	Ethyl Acetate	Methanol
Alkaloids	–	–	+++	++
Flavonoids	–	–	++	+++
Phenols	–	–	–	++
Saponins	–	–	++	++
Steroids	–	+	–	++
Tannins	–	–	+	+++
Terpenoids	+	+	++	++

'+++' indicates strong presence, '++' moderate, '+' slight presence, and '—' absence

Table 2: Quantitative estimation of secondary metabolites of *D. bicameratum* leaf extracts

D. bicameratum leaf Solvent extract	TAC (mg/g) dry weight	TPC (µg/mg of gallic acid equivalent)	TFC (µg/mg of quercetin equivalent)
Ethyl acetate leaf extract	1.11±0.001	186.41±0.90	11.58±0.07
Methanol leaf extract	1.45±0.012	343.17±0.31	49.23±0.05

TAC=Total alkaloid content, TPC=Total phenol content, TFC=Total flavonoid content. The data given in the table are the mean±S.D. of triplicate experiments

Assessment of key secondary metabolites

In this study, higher levels of alkaloid, phenol and flavonoid content were recorded in methanol leaf extract in comparison to ethyl acetate leaf extracts (Table 2). The extraction efficiency of methanol can be ascribed to its polarity, which enables it to dissolve a broader spectrum of secondary metabolites. Alkaloids and several phenolic substances possess functional groups which show better solubility in methanol than in ethyl acetate, resulting in high quantitative yields. Similar observations have been documented in previous phytochemical investigations, where methanol reliably extracted better amounts of bioactive metabolites compared to other solvents (Felhi *et al.,* 2017).

Antioxidant potential of D. bicameratum leaf extracts

Antioxidants can counteract ROS (reactive oxygen species), thereby decreasing oxidative damage and contributing to cancer prevention (Kozlov *et al.,* 2024). Plant-derived antioxidants are well-acknowledged for their capability to scavenge free radicals and mitigate oxidative stress (Szymanska *et al.,* 2016). In the current investigation, *D. bicameratum* leaf extracts presented dose-dependent radical scavenging (Table 3). The methanol leaf extract at 120 µg/mL exhibited the highest inhibition percentage (85.80±0.73 %), followed by ethyl acetate leaf extract (82.97±0.48%). The IC_{50} value of methanol leaf extract is 17.46±0.60 µg/mL (Table 3, & Figure 1). The standard ascorbic acid showed an IC_{50} value of 9.8±0.38 µg/mL. Both the leaf extracts displayed a strong concentration-dependent increase in radical scavenging. The methanol leaf extract showed a strong positive correlation (r=0.9871, p=0.00235), while the other leaf extract indicated an almost perfect linear relationship with concentration (r=0.9905, p=0.000133). Therefore, these results confirm that the leaf extracts possess potent, concentration-driven radical scavenging ability.

The FRAP assay revealed that *D. bicameratum* leaf extracts showed varying reduction percentages (Table 4). In this assay, both leaf extracts showed a strong dose-dependent increase in reducing power. The ethyl acetate leaf extract showed a high positive correlation with concentration (r=0.9598, p=0.00236), while the methanol leaf extract displayed an even stronger relationship (r=0.9667, p=0.00169). These significant correlations confirm that the ferric-reducing ability of the extracts increases in proportion to their concentration. Both these extracts showed better antioxidant activity at 120 µg/mL in comparison to previous doses. The methanol leaf extract displayed a good EC_{50} value, i.e., 101.12±0.94 (Table 4 & Figure 2), which indicates higher antioxidant efficacy compared

Table 3: DPPH radical scavenging ability of *D. bicameratum* leaf extracts

D. bicameratum	Concentration (µg/mL)	Absorbance			% of inhibition				IC_{50} values µg/mL concentration	Pearson correlation coefficient (r) and p value (p)
		I	II	III	%-I	%-II	%-III	Mean±SD		
Methanol leaf Extract	Control	0.315	0.313	0.316	-	-	-	-	17.46±0.60	r=0.9871 p=0.00235
	20	0.168	0.165	0.170	46.66	47.28	46.20	46.71±0.54		
	40	0.124	0.121	0.127	60.63	61.34	59.81	60.59±0.76		
	60	0.098	0.096	0.099	68.88	69.32	68.67	68.95±0.33		
	80	0.076	0.074	0.078	75.87	76.35	75.31	75.84±0.52		
	100	0.059	0.055	0.060	81.26	82.42	81.01	81.56±0.75		
	120	0.045	0.042	0.047	85.71	86.58	85.12	85.80±0.73		
Ethyl acetate Leaf Extract	Control	0.358	0.357	0.360	-	-	-	-	29.22±0.61	r=0.9905 p=0.000133
	20	0.210	0.208	0.213	41.34	41.73	40.83	41.3±0.45		
	40	0.152	0.150	0.155	57.54	57.98	56.94	57.48±0.52		
	60	0.124	0.122	0.127	65.36	65.82	64.72	65.3±0.55		
	80	0.103	0.100	0.105	71.22	71.98	70.83	71.34±0.58		
	100	0.086	0.078	0.082	75.97	78.15	77.22	77.11±1.09		
	120	0.061	0.059	0.063	82.96	83.47	82.50	82.97±0.48		

Table 4: FRAP assay of *D. bicameratum* leaf extracts

D. bicameratum	Concentration (µg/mL)	Absorbance			% of Fe^{3+}reduction				EC_{50} values µg/ml Concentration	Pearson correlation coefficient (r) and p value (p)
		I	II	III	%-I	%-II	%-III	Mean±SD		
Ethyl acetate leaf extract	Control	0.148	0.146	0.145	-	-	-	-	123.57±0.74	r=0.9598 p=0.00236
	20	0.156	0.153	0.150	5.12	4.57	3.33	4.34±0.91		
	40	0.189	0.185	0.180	21.69	21.08	19.44	20.73±1.16		
	60	0.213	0.209	0.205	30.51	30.14	29.26	29.97±0.64		
	80	0.232	0.226	0.223	36.20	35.39	34.97	35.52±0.62		
	100	0.251	0.245	0.242	41.03	40.40	40.08	40.50±0.48		
	120	0.274	0.266	0.262	45.98	45.11	44.65	45.24±0.67		
Methanol leaf extract	Control	0.120	0.118	0.122	-	-	-	-	101.12±0.94	r=0.9667 p=0.00169
	20	0.144	0.139	0.149	16.66	15.10	18.12	16.62±1.51		
	40	0.181	0.176	0.187	33.70	32.95	34.75	33.80±0.90		
	60	0.201	0.195	0.208	40.29	39.48	41.34	40.37±0.93		
	80	0.219	0.215	0.228	45.20	45.11	46.49	45.6±0.77		
	100	0.233	0.226	0.243	48.49	47.78	49.79	48.68±1.01		
	120	0.257	0.248	0.262	53.30	52.41	53.43	53.04±0.55		

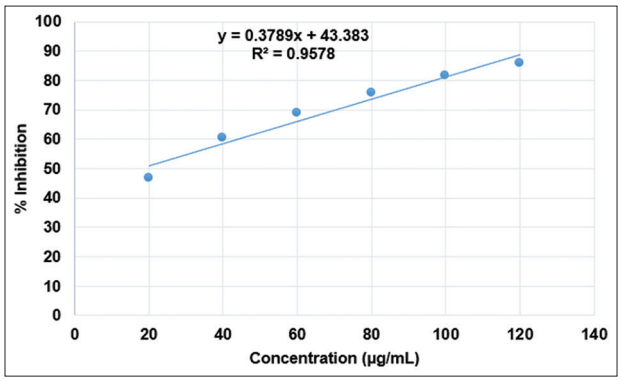

Figure 1: DPPH activity of the *D. bicameratum* methanol leaf extract: % Inhibition plotted against leaf extract concentration

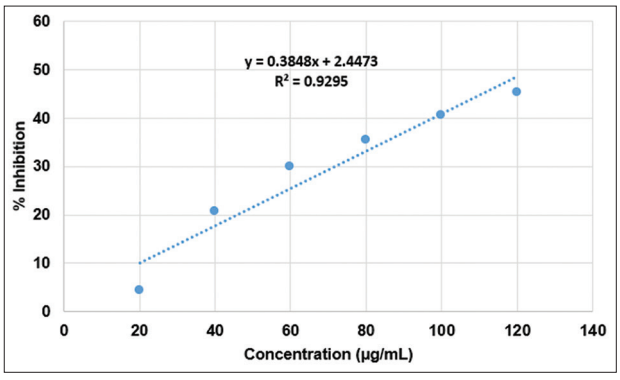

Figure 2: FRAP dose–response curve of the *D. bicameratum* methanol leaf extract showing ferric-reducing activity and EC_{50}

to the ethyl acetate leaf extract. Consequently, the ethyl acetate leaf extract required a higher concentration to attain 50% reduction, indicating comparatively weaker reducing activity. The positive control ascorbic acid displayed the strongest EC_{50} value (15.20±0.34) among all the tested samples.

In the present investigation, the *D. bicameratum* methanol leaf extract exhibited greater antioxidant potential than the ethyl acetate leaf extract in both DPPH and FRAP assays. Generally, the antioxidant property of the plant extracts is linked to the solvents utilised for the extraction of phytocompounds because the chemical compounds with antioxidant potential dissolve differentially in different polarities of solvents (Boeing *et al.,* 2014). The antioxidant-rich nature of the methanol leaf extract can be ascribed to its higher alkaloid, phenol, and flavonoid contents. These metabolites are well known to contribute to radical scavenging and reducing power. Though the ascorbic acid exhibited good IC_{50}/EC_{50} values, signifying its greater potency, the methanol leaf extract still showed appreciable antioxidant capacity.

Table 5: Cytotoxic activity of *D. bicameratum* leaf extracts on breast cancer cells (MDA-MB-231)

D. Bicameratum	Control OD Mean	Concentration (µg/mL)	Absorbance at 570 nm			% of cell death			% of cell death Mean±SD	% Live cell	IC_{50} µg/mL	Pearson correlation coefficient (r) and p value (p)
			OD 1	OD 2	OD 3							
Ethyl acetate leaf extract	0.360	Control (Untreated)	0.361	0.359	0.362	-	-	-	-	100	7.52±0.80	r=0.979 p=0.00015
		1.56	0.256	0.253	0.259	28.88	29.72	28.05	28.88±0.83	71.12		
		3.125	0.198	0.195	0.201	45.00	45.83	44.16	44.99±0.83	55.01		
		6.25	0.176	0.172	0.178	51.11	52.22	50.55	51.29±0.84	48.71		
		12.5	0.155	0.151	0.157	56.94	58.05	56.38	57.12±0.84	42.88		
		25	0.101	0.099	0.104	71.94	72.50	71.11	71.85±0.58	28.15		
		50	0.059	0.055	0.060	83.61	84.72	83.33	83.88±0.73	16.12		
		100	0.028	0.023	0.030	92.22	93.61	91.66	92.49±1.00	7.51		
Methanol leaf extract	0.332	Control (Untreated)	0.331	0.333	0.332	-	-	-	-	100	3.84±0.62	r=0.974 p=0.0259
		1.56	0.226	0.229	0.227	31.92	31.02	31.62	31.52±0.45	68.48		
		3.125	0.178	0.182	0.180	46.38	45.18	45.78	45.78±0.60	54.22		
		6.25	0.155	0.160	0.157	53.31	51.80	52.71	52.60±0.76	47.4		
		12.5	0.126	0.131	0.129	62.04	60.54	61.14	61.24±0.75	38.76		
		25	0.086	0.090	0.088	74.09	72.89	73.49	73.49±0.60	26.51		
		50	0.039	0.043	0.041	88.25	87.04	87.65	87.64±0.60	12.36		
		100	0.009	0.013	0.011	97.28	96.08	96.68	96.68±0.60	3.32		

Earlier research studies reported that phenol derivatives can reduce and decolourise DPPH free radicals (Tiveron *et al.,* 2012). The solvent extracts of *D. aqueum* (Mukherjee *et al.,* 2012), *D. speciosum* (Moretti *et al.,* 2013), *D. amoenum* (Paudel *et al.,* 2015), *D. signatum* (Chimsook, 2016) and *D. longicornu* (Paudel *et al.,* 2017) are reported to have antioxidant properties, related to the high phenol and flavonoid content. Peng *et al.* (2024) reported that leaf extracts of *D. chrysotoxum* and *D. denneanum* showed the highest antioxidant activities. Studies by Luo *et al.* (2023) indicated that flavonoid content is mainly contributing to the antioxidant activity of *D. officinale* than phenolic content. Therefore, the results of the present study and previous research reports suggest that genus *Dendrobium* is a rich reservoir of antioxidant compounds.

Correlation of anticancer activity with antioxidant potential

Cancer is a disease associated with modern living and lifestyle, for which modern medicine has very little to offer. Very few are aware that this disease is curable if detected early and also preventable if proper precautions are taken. The real answer lies with the revival of traditional herbal medicine and the search for more biochemical compounds which can selectively arrest cell divisions in abnormally growing cells and tissues in the human body. Breast cancer is one of the most predominant cancers among women worldwide, and current treatment options primarily rely on chemotherapy and radiotherapy, which are often associated with significant adverse side effects. Therefore, there is an urgent need to explore plant-derived bioactive compounds. In the recent past, Cirrhopetalanthrin, which has shown cytotoxicity against various human cancer cell lines, was isolated from *Cremastra appendiculata* (Xia *et al.,* 2005).

The results of the MTT assay revealed that *D. bicameratum* methanolic leaf extract strongly suppressed cell proliferation in comparison to the ethyl acetate leaf extract (Table 5). Treatment of cancer cells with leaf extracts

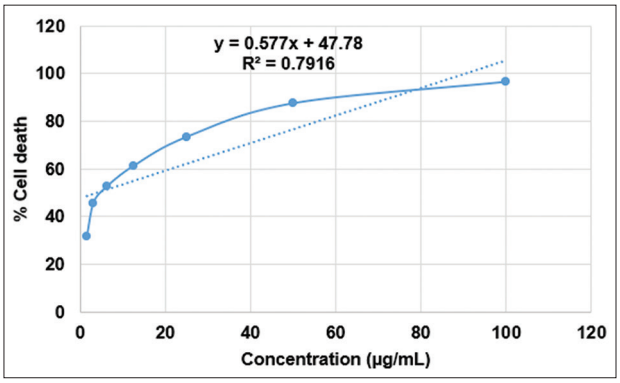

Figure 3: Dose-dependent cytotoxic effect of *D. bicameratum* methanol leaf extract on MDA-MB-231 breast cancer cells

induced marked morphological alterations, including reduced cell size and volume, cell shrinkage, membrane blebbing, chromatin condensation, nuclear fragmentation, and the formation of apoptotic bodies, indicating that the leaf extracts effectively triggered apoptotic cell death. The ethyl acetate leaf extract displayed perfect correlation between leaf concentration and cytotoxicity (r=0.979, p=0.00015), while the methanol leaf extract also exhibited a strong and significant positive relationship (r=0.974, p=0.0259). The *D. bicameratum* methanol leaf extract at 100 µg/mL resulted in a significant cell death rate (96.68%) with a low percentage of viable cells (3.32%). The cell death percentage increased along with a rise in leaf extract concentration. The IC_{50} value of the methanol leaf extract was 3.84±0.62 µg/mL (Table 5 & Figure 3), implying significant cytotoxic potential, whereas the ethyl acetate leaf extract showed 7.52±0.80 µg/mL. The leaf extracts exhibited a marked cytotoxic potential, showing good IC_{50} values, which were comparatively close to the standard drug cisplatin (0.92 µg/mL). Although the drug cisplatin remained more active, the leaf extracts showed slightly lower potency compared to it, indicating the occurrence of bioactive compounds with chemotherapeutic relevance.

In this study, flavonoids and phenolic compounds are reported from both leaf extracts. Flavonoids exhibit strong antioxidant properties by inhibiting oxidative and hydrolytic enzymes, and play a crucial role in free radical scavenging and anticancer activity (Alsabri *et al.,* 2013). The leaf extracts tested here showed anticancer properties due to the presence of phenolics and nitrogen compounds (Sharifi-Rad *et al.,* 2023). The significant anticancer activity exhibited by the methanol leaf extract may be attributed to its high antioxidant capacity, as indicated by the good IC_{50} and EC_{50} values recorded in the DPPH and FRAP assays. The results of this investigation align with the research reports of Li *et al.* (2007), who found that phenolic-rich herbal extracts showed good antioxidant properties and growth inhibition against two cancer cell lines (A549 and MCF-7). Their studies also reported a positive correlation between antioxidant and anticancer effects. This suggests that phenolic compounds have a central role in silencing cancer cell proliferation. Similarly, in the current study, *D. bicameratum* leaf extracts with higher phenolic, flavonoid, and alkaloid content, particularly the methanol leaf extract, showed superior antioxidant activity and enhanced anticancer potential. Further, the cytotoxicity of the leaf extracts is enhanced due to the synergetic effect of all phytocompounds present in the extracts. The antioxidant and anticancer properties of the extract may be attributed to the combined effect of multiple bioactive compounds, facilitated by enhanced reaction kinetics and improved bioavailability of the active constituents (Long *et al.,* 2015).

Numerous phenolic substances have dual biological roles, acting as free-radical scavengers and cytotoxins, thus disrupting oxidative signalling pathways that support cancer cell proliferation. Since oxidative stress has an important role in tumour development and survival, plant extracts with higher antioxidant potential are frequently documented to induce apoptosis, cell-cycle arrest and mitochondrial dysfunction in cancer cells. The cytotoxic nature of the leaf extracts may be influenced by their rich antioxidant compounds, which can modulate redox balance within malignant cells. However, more mechanistic studies and bioassay-guided fractionation are needed to confirm whether the anticancer activity is directly correlated with antioxidant phytochemicals or arises from independent cytotoxic metabolites.

Many researchers across the globe have been screening the orchids for the presence of phytochemicals that are useful to mankind. Haridas *et al.* (2016) evaluated the anticancer activity of *Malaxis rheedii* and found that its methanolic extract had better anticancer activity against MCF-7 than HeLa cell lines. Bhatt *et al.* (2018) reported that the extracts of *Eulophia nuda* tubers exhibited cytotoxicity on the MCF-7 breast cancer cells. Numerous *Dendrobium* species showed noteworthy cytotoxic effects against various cancer cell types, signifying the genus as a repository of anticancer agents (Paudel *et al.,* 2018). Shukla *et al.* (2022) reported that phytocompounds from various orchid species, such as *Dendrobium longicornu*, *D. transparens*, *Rhynchostylis retusa*, *Vanda cristata* and *Anoectochilus formosanus,* show anticancer properties through diverse mechanisms. Therefore, previous research studies and findings of the present investigation suggest that the cytotoxic activity displayed by the *D. bicameratum* leaf extracts may similarly be attributed to bioactive compounds capable of interfering with cancer cell growth and survival.

Conclusion

The leaf extracts of *D. bicameratum,* particularly the methanol leaf extract, contain a rich diversity of phytochemicals, which are responsible for their strong antioxidant and cytotoxic activities. The presence of phenolic, flavonoid, and other bioactive compounds likely contributes to both their antioxidant and anticancer potential. These findings validate the traditional use of *D. bicameratum* and authenticate its potential as a natural source of antioxidant and anticancer compounds. Future research, directed toward the isolation and structural elucidation of bioactive constituents, supported by *in vivo* studies, is strongly recommended.

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Author contributions

Amzad Basha Kolar: Conceptualization, Data curation, Investigation, Formal analysis, Writing – original draft. M. Rahamtulla: Investigation, Methodology. B. Sandhya: Formal analysis, Validation, Visualization. Diptendu Sarkar: Supervision, Writing – review & editing. All authors approved the final manuscript.

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