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Evaluation of Antioxidant Potential and Selective Cytotoxicity of *Trichosanthes tricuspidata* Lour.

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ABSTRACT

Plant-derived antioxidants are of particular interest due to their ability to neutralize reactive oxygen species involved in cancer initiation and progression. This study evaluated the antioxidant and anticancer potential of leaf and bark extracts of the endangered medicinal plant *Trichosanthes tricuspidata* Lour. using *in vitro* assays. Antioxidant activity was assessed by DPPH radical scavenging and ferric reducing antioxidant power (FRAP) assays, while cytotoxic efficacy was determined against the human colorectal adenocarcinoma cell line HCT-116 using the MTT assay. Among the tested extracts, the methanolic bark extract exhibited the strongest antioxidant activity, with IC_{50} values of 35.80±4.04 µg/ml (DPPH) and 43.22±0.20 µg/ml (FRAP), approaching the activity of ascorbic acid. The methanolic leaf extract also demonstrated substantial antioxidant potential (DPPH IC_{50} = 44.38 ± 4.20 µg/ml). Cytotoxicity results revealed a concentration-dependent inhibition of HCT-116 cell proliferation, with the methanolic leaf extract showing higher anticancer efficacy (IC_{50} = 129.4±2.11 µg/ml) and inducing over 90% growth inhibition at 500 µg/mL. GC-MS analysis of methanolic extracts identified several bioactive constituents, including 1,4-benzenedicarboxylic acid bis(2-ethylhexyl) ester, stigmast-7-en-3-ol, and matairesinol, which may contribute to the observed bioactivities. These findings suggest that *T. tricuspidata*, particularly its methanolic leaf extract, holds promise as a natural source of antioxidant and anticancer compounds for colorectal cancer management.

Key words: Antioxidant activity, colorectal adenocarcinoma, cytotoxicity (MTT assay), HCT-116 cell line, GC-MS spectroscopy

INTRODUCTION

Plants constitute a rich and invaluable source of bioactive compounds, particularly phenolic constituents such as flavonoids, phenolic acids, tannins and lignans, which have garnered significant scientific attention due to their health-promoting properties. These phenolic compounds exhibit strong antioxidant activity by neutralizing reactive oxygen species (ROS), including free radicals, peroxides, singlet oxygen, hydroxyl radicals and nitric oxide radicals, thereby reducing oxidative stress implicated in the development of chronic diseases such as cardiovascular disorders, cancer and neurodegenerative conditions. Numerous studies have established a positive correlation between the phenolic content of plant extracts and their free radical scavenging potential, emphasizing the crucial role of phenolics in oxidative stress mitigation. The therapeutic efficacy of medicinal plants arises from their diverse phytochemical composition, including alkaloids, flavonoids, terpenoids and glycosides, which not only contribute to antioxidant defence but also possess antimicrobial, anti-inflammatory and anticancer properties. Growing concerns over antimicrobial resistance and the adverse effects of synthetic drugs have further reinforced interest in plant-derived compounds.

Cancer remains one of the leading causes of mortality worldwide, driven by complex genetic and molecular alterations that result in uncontrolled cell proliferation, invasion and metastasis. Among the various malignancies, colorectal cancer is one of the most common and lethal cancers globally, affecting both men and women and posing a significant public health burden. Colorectal cancer primarily arises from the epithelial cells of the colon and rectum and is often associated with lifestyle factors, dietary habits, chronic inflammation and genetic predisposition. Plant-derived bioactive compounds, especially phenolics and

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other phytochemicals, have demonstrated significant anticancer potential through mechanisms such as induction of apoptosis, inhibition of cell proliferation, modulation of signalling pathways, suppression of angiogenesis and antioxidant activity. Compared to conventional chemotherapeutic agents, phytochemicals offer the advantages of lower toxicity and multi-targeted action, making medicinal plants a promising resource for the development of future colorectal cancer preventive and therapeutic interventions.

Trichosanthes tricuspidata Lour. (Cucurbitaceae), a perennial climbing vine of notable ethnomedicinal importance, is widely distributed across tropical and sub-tropical regions of South, South-east Asia and Northern Australia, typically inhabiting forest margins and disturbed habitats at elevations between 1200-2300 m (Rekha *et al*., 2021). Morphologically, the plant exhibits deeply lobed palmately divided leaves, white fimbriate flowers, and globose fruits that mature from green to bright red, enclosing flattened, winged seeds (Pradheep *et al*., 2021). Traditionally, various parts of the plant have been employed in Ayurveda, Siddha, and folk medicine for treating respiratory and gastrointestinal disorders, epilepsy, smallpox and asthma, while fruits and seeds are valued for anthelmintic and mosquito-larvicidal activities (Rekha *et al*., 2021; Duvey *et al*., 2025). Phytochemical investigations reveal that *T. tricuspidata* is rich in alkaloids, flavonoids, glycosides, saponins, terpenoids, tannins and phenolic compounds, which collectively contribute to its broad pharmacological profile (Abhiram *et al*., 2024). Recent studies demonstrate potent antioxidant and enzyme-inhibition activity, with methanolic and ethanolic extracts showing high total phenolic and flavonoid content and strong á-amylase inhibition, supporting its antidiabetic potential (Kulandaivel *et al*., 2023). In this study, the less investigated *T. tricuspidata* plant leaf and bark extracts were examined for phytochemical, antibacterial, anti-oxidant, anti-inflammatory and anticancer activities in HCT116 cells.

MATERIALS AND METHODS

Leaves and bark of *T. tricuspidata* were collected manually in January 2024 from the Paderu forest range, Kommula Mamidi village, Andhra Pradesh, India (18°002 55.5483 N, 82°292 43.2963 E; 956 m). The plant material was taxonomically authenticated at Andhra University (Herbarium No. AUV: 26646). Samples were shade-dried at ~32°C, pulverized, sieved (0.5 mm) and stored under sterile conditions. Soxhlet extraction was performed using 100 g of powdered material with methanol, acetone, chloroform, petroleum ether and water. Extracts were concentrated and preserved for further analyses.

Methanolic leaf and bark extracts were subjected to GC-MS analysis at SAIF, IIT Madras, using an Agilent 8890 GC coupled with a 5977 MSD. Samples (1 µl) were injected into a capillary column (30 m × 250 µm × 0.25 µm). Helium served as the carrier gas at 1.2 ml/min, with EI ionization at 70 eV. Compounds were identified based on retention times and mass spectra by comparison with the NIST 2017 library using OpenLab CDS 2.5.

Antibacterial efficacy was evaluated by the agar well diffusion method against *Staphylococcus aureus* (MTCC 96), *Streptococcus mutans* (MTCC 497), *Escherichia coli* (MTCC 443) and *Pseudomonas aeruginosa* (MTCC 424). Bacterial cultures were prepared in nutrient broth and spread onto nutrient agar plates. Wells (5 mm) were loaded with extracts (10, 5 and 2.5 mg in 10% DMSO). Streptomycin and penicillin (100 µg/ml) were used as positive controls for Gram-negative and Gram-positive bacteria, respectively, while 10% DMSO served as the negative control. After incubation at 37°C for 24 h, zones of inhibition were measured. Experiments were conducted in triplicate and expressed as mean±SD.

Antioxidant potential was assessed using DPPH and FRAP assays. For DPPH, extracts (100-500 µg/ml) were evaluated for radical scavenging activity by measuring absorbance at 517 nm, with ascorbic acid as the standard. The IC_{50} values were calculated from dose-response curves. In the FRAP assay, extracts were mixed with freshly prepared FRAP reagent (1:30), incubated for 30 min in the dark, and absorbance was recorded at 593 nm. Results were expressed as percentage ferric reducing activity using ascorbic acid as the reference. Percentage inhibition was calculated using the formula:

$$\% \text{ inhibition} = [(A_0 - A_1)/A_0] \times 100,$$

where A_0 is control absorbance and A_1 is sample absorbance.

The cytotoxic effect of methanolic extracts was evaluated against HCT-116 colorectal cancer cells using the MTT assay. Cells (1 × 10t /well) were seeded in 96-well plates and cultured in RPMI-1640 supplemented with 10% FBS and antibiotics at 37°C in 5% CO, . After overnight incubation, cells were treated with varying extract concentrations. Doxorubicin (1 µg/ml) served as the positive control. Following 24 h treatment, MTT (0.5 mg/ml) was added and incubated for 4 h. Formazan crystals were solubilized with DMSO, and absorbance was measured at 570 nm. Cell viability (%) and IC_{50} values were derived from dose-response curves.

RESULTS AND DISCUSSION

GC-MS analysis of the methanolic extracts of *T. tricuspidata* leaf and bark was carried out to elucidate the phytochemical constituents responsible for the observed biological activities. Methanol was selected as the extraction solvent due to its ability to recover a broad range of polar and moderately non-polar secondary metabolites, as widely reported in phytochemical investigations (Satapathy *et al*., 2025). The chromatographic results revealed a relatively simple chemical profile, with two compounds detected in the leaf extract and four compounds identified in the bark extract (Figs. 1, 2; Table 1). In the leaf extract, 1,4-benzenedicarboxylic acid, bis(2-ethylhexyl) ester was the predominant constituent, accounting for 96.96% of the total chromatographic area. A minor component, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (3.04%), was also detected and is known for its antioxidant and antibacterial potential.

Similarly, the bark extract was dominated by 1,4-benzenedicarboxylic acid, bis(2-ethylhexyl) ester (95.88%), confirming its abundance in

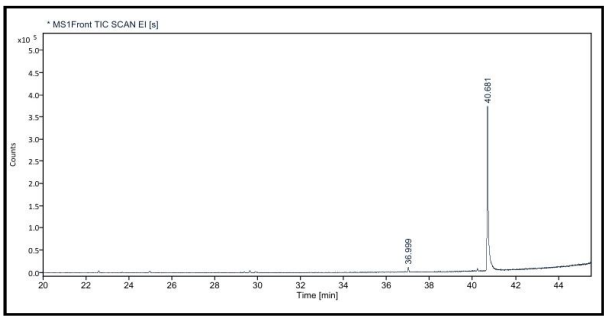

Fig. 1. GC-MS chromatogram of *T. tricuspidata* leaf methanol extract.

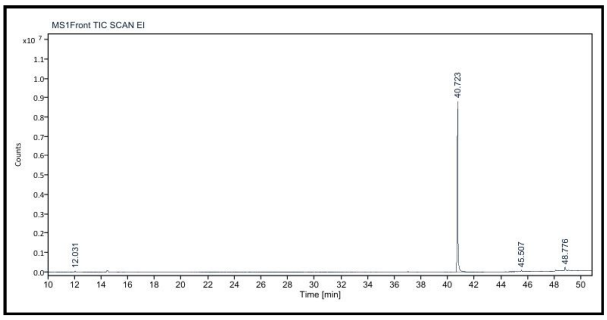

Fig. 2. GC-MS chromatogram of *T. tricuspidata* bark methanol extract.

both the plant parts. Additional constituents identified in lower proportions included stigmast-7-en-3-ol (2.89%), a phytosterol reported for its anti-inflammatory, cholesterol-lowering, anticancer properties and matairesinol (0.85%), a lignan with well-documented antioxidant, phytoestrogenic, and anticancer activities. A trace amount of DL-proline, 5-oxo-, methyl ester (0.38%) was also observed, which may be linked to cellular stress response mechanisms. Collectively, the predominance of bioactive esters along with the presence of phytosterols and lignans provided a phytochemical basis for the therapeutic potential of *T. tricuspidata*.

The antibacterial activity of *T. tricuspidata* leaf and bark extracts prepared using methanol, acetone and water was assessed against

Table 1. Identified compounds from *T. tricuspidata* leaf and bark methanol extracts through GC-MS

S. No	Name of the compound	RT minutes	Mol. weight	Mol. formula	Area%
Leaf					
1.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	36.999	330.5	$C_{19}H_{38}O_4$	3.04
2.	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	40.681	390.6	$C_{24}H_{38}O_4$	96.96
Bark					
3.	DL-Proline, 5-oxo-, methyl ester	12.031	143.14	$C_6H_9NO_3$	0.38
4.	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	40.723	390.6	$C_{24}H_{38}O_4$	95.88
5.	Matairesinol	45.507	358.4	$C_{20}H_{22}O_6$	0.85
6.	Stigmast-7-en-3-ol, (3â,5á,24S)	48.776	414.7067	$C_{29}H_{50}O$	2.89

Escherichia coli, *Pseudomonas aeruginosa*, *Streptococcus mutans* and *Staphylococcus aureus* at concentrations of 10, 5 and 2.5 mg (Tables 2 and 3). Antibacterial efficacy varied significantly with solvent polarity, extract concentration and bacterial species, with methanolic extracts showing superior activity compared to aqueous and acetone extracts. Methanolic leaf extracts exhibited strong antibacterial activity, particularly against Gram-positive bacteria. Maximum inhibition was recorded against *S. aureus* (26.3±0.57 mm at 10 mg), followed by *S. mutans* (25±1 mm), with activity decreasing in a concentration-dependent manner. Moderate inhibition was observed against *P. aeruginosa* and *E. coli*, while no activity was detected against *E. coli* at the lowest concentration. Aqueous leaf extracts showed only mild to moderate activity, which declined sharply at lower concentrations, whereas acetone leaf extracts were completely inactive, indicating poor extraction of antibacterial constituents. Similar solvent-dependent trends favouring methanol have been reported for the extraction of phenolics and flavonoids (Satapathy *et al*., 2025).

Bark extracts exhibited stronger and broader-spectrum antibacterial activity compared to leaf extracts. Methanolic bark extract showed the highest efficacy, with maximum inhibition against *S. aureus* (25.6±0.57 mm), followed by *S. mutans*, *P. aeruginosa* and *E. coli* at 10 mg. Notably, significant inhibition persisted even at 2.5 mg, indicating the presence of potent antimicrobial compounds. Acetone bark extracts showed moderate activity, mainly against *S. aureus* and *P. aeruginosa*, but were less effective than methanolic extracts and largely inactive at lower concentrations. These results align with earlier studies reporting bark tissues as richer sources of antimicrobial constituents than leaves (Prabhakara Rao *et al*., 2025). Gram-positive bacteria were more susceptible than Gram-negative strains, likely due to the restrictive outer membrane of Gram-negative bacteria limiting phytochemical penetration. Although streptomycin produced larger inhibition zones, the methanolic bark extract of *T. tricuspidata* exhibited comparable activity, particularly against *S. aureus*. These findings highlight *T. tricuspidata*, especially its bark, as a promising natural source of antibacterial agents.

Table 2. Antibacterial activity of different extracts (Me, Ac and Aq) of *T. stricuspida* leaf at the dosages of 10, 5 and 2.5 mg

Extract	*E. coli*			*P. aureginosa*			*S. mutans*			*Staphylococcus aureus*		
	10 mg	5 mg	2.5 mg	10 mg	5 mg	2.5 mg	10 mg	5 mg	2.5 mg	10 mg	5 mg	2.5 mg
Me	13.3±0.57	11±1	-	12.3±0.57	9±1	6±1	25±1	22.3±0.57	19.6±0.57	26.3±0.57	20.6±0.57	13±1
Ac	-	-	-	-	-	-	-	-	-	-	-	-
Aq	14±1	8.3±0.57	7±1	14±1	12.3±0.57	9±1	12.3±0.57	9.6±0.57	8±1	14±1	9.3±0.57	8±1
+ Ve		32.2±1.23			31.3±0.57			28±1			27.6±1.52	

Me stands for methanol extract, Ac for acetone extract, Aq for aqueous extract and "-" for no zone of inhibition.

Table 3. Antibacterial activity of different extracts (Me, Ac and Aq) of *T. stricuspida* bark at the dosages of 10, 5 and 2.5 mg

Extract	*E. coli*			*P. aureginosa*			*S. mutans*			*Staphylococcus aureus*		
	10 mg	5 mg	2.5 mg	10 mg	5 mg	2.5 mg	10 mg	5 mg	2.5 mg	10 mg	5 mg	2.5 mg
Me	18.25±0.75	14.3±0.57	12±1	18.3±0.75	17±1	16±0	24±1	18.3±0.57	12±1	25.6±0.57	19±1	14.3±0.57
Ac	13.3±0.57	11±1	-	16.3±0.57	13±1	7±1	15±1	11.3±0.57	-	18±1	13±0	6.3±0.57
Aq	-	-	-	-	-	-	-	-	-	-	-	-
+ Ve		32.2 ± 1.23			31.3± 0.57			28 ± 1			27.6 ± 1.52	

Me stands for methanol extract, Ac for acetone extract, Aq for aqueous extract and "-" for no zone of inhibition.

The antioxidant capacity of *T. tricuspidata* leaf and bark extracts was quantitatively assessed using DPPH and FRAP assays, with results expressed as IC_{50} values (ìg/ml) (Table 4). Among the various solvents tested, the methanolic extracts demonstrated the most potent radical scavenging and reducing power. Specifically, the methanolic bark extract yielded the lowest IC_{50} values in both the DPPH (35.80±4.04 ìg/ml) and FRAP (43.22±0.20 ìg/ml) assays, closely approaching the efficacy of the ascorbic acid control (19.58±1.31 ìg/ml and 25.48±1.31 ìg/ml, respectively). Methanolic leaf extracts also showed significant antioxidant activity, outperforming acetone, chloroform and petroleum ether counterparts. This superior performance of methanol as a solvent suggests a high concentration of polar antioxidant compounds, such as polyphenols, which are known to stabilize free radicals through electron or hydrogen atom donation.

In contrast, the non-polar and highly polar aqueous solvents exhibited substantially lower antioxidant potential. Petroleum ether and water extracts recorded the highest IC_{50} values, often exceeding 300 ìg/ml indicating limited presence for bioactive hydrogen donating constituents. For instance, the aqueous leaf extract showed a DPPH IC_{50} of 348.36±66.26 ìg/ml, which was nearly 10 times less potent than the methanolic version. Across both the assays, the bark generally exhibited slightly higher antioxidant efficiency than the leaves, particularly in the acetone and methanol preparations. These findings correlate with the previously observed antibacterial trends, suggesting that the bark of *T. tricuspidata* serves as a more concentrated source of redox-active secondary metabolites compared to the leaf tissue.

The superior performance of methanolic bark extract, as evidenced by its low IC... € values in both DPPH and FRAP assays, closely comparable to ascorbic acid, was consistent with previous studies reporting methanol as an efficient solvent for extracting phenolic antioxidants from medicinal plants (Satapathy *et al*., 2025). The enhanced antioxidant capacity of bark over leaf tissues aligned with earlier findings that bark often accumulates higher concentrations of polyphenols, flavonoids and other redox-active secondary metabolites responsible for neutralizing free radicals (Shalini and Ilango, 2021). Conversely, the weak activity observed in aqueous extracts corroborates existing literature indicating that non-polar solvents and highly polar aqueous systems are less effective in solubilizing antioxidant phytochemicals with hydrogen- or electron-donating capabilities.

The cytotoxic potential of methanolic leaf and bark extracts of *T. tricuspidata* was evaluated against the HCT-116 human colon cancer cell line using the MTT assay (Figs. 3 and 4). Both extracts exhibited concentration-dependent reductions in cell viability, although notable differences in potency were observed between plant parts. The methanolic leaf extract demonstrated strong cytotoxic activity at higher concentrations. At 500 µg/ml, cell viability was reduced to approximately 9%, indicating more than 90% growth inhibition, and the effect remained substantial at 250 µg/ml, where viability declined to about 36%. Moderate cytotoxicity was observed at 100 µg/ml, with nearly 50% inhibition of cell growth, whereas lower concentrations (50 and 25 µg/ml) produced minimal effects on cell viability. Notably, the leaf extract at the highest concentration exhibited greater cytotoxic efficacy than the positive control. The IC... € value for the methanolic leaf extract was calculated as 129.4±2.11 µg/ml, reflecting moderate anticancer potency against HCT-116 cells.

In contrast, the methanolic bark extract of *T. tricuspidata* showed comparatively weaker cytotoxic activity. A marked reduction in cell

Table 4. IC_{50} values (µg/ml) of DPPH and FRAP assays in various extracts of *T. tricuspidata*

Sample extracts	DPPH IC_{50} (Leaf)	DPPH IC_{50} (Bark)	FRAP IC_{50} (Leaf)	FRAP IC_{50} (Bark)
Water	348.36±66.26	298.15±59.23	291.36±66.26	324.36±42.26
Methanol	44.38±4.20	35.80±4.04	90.38±4.20	43.22±0.20
Acetone	126.77±2.64	117.11±1.61	108.77±2.64	73.86±1.64
Chloroform	242.08±15.24	208.33±5.70	173.08±15.24	202.36±14.24
Petroleum ether	331.64±15.32	334.26±13.66	292.64±15.32	326.64±16.32
Control (Ascorbic acid)	19.58±1.31		25.48±1.31	

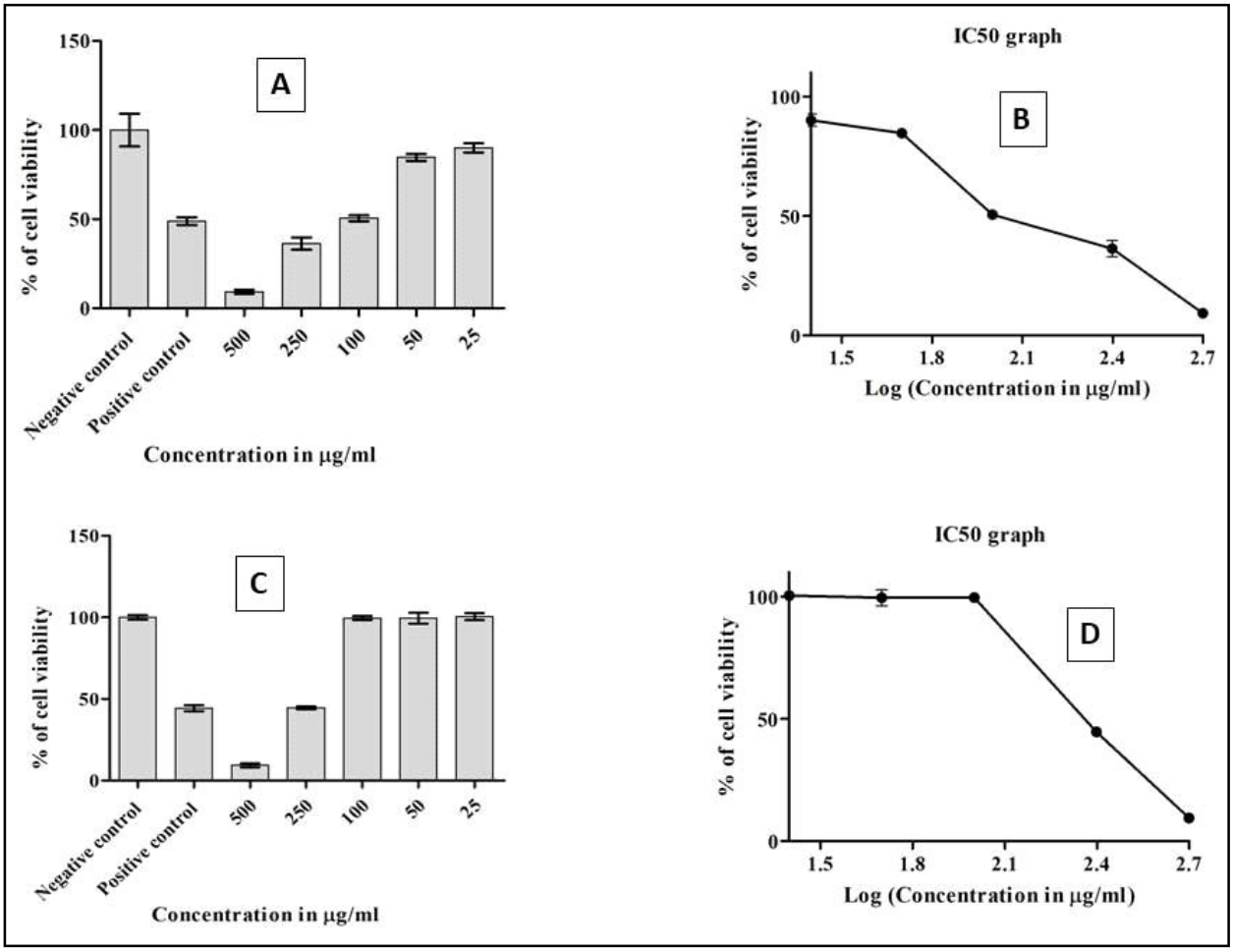

Fig. 3. (a) Leaf methanol extract's cell viability percentage on HCT-116 cell line, (B) Leaf methanol extract's IC_{50} calibration graph on HCT-116 cell line, (C) Bark methanol extract's cell viability percentage on HCT-116 cell line and D) Bark methanol extract's IC_{50} calibration graph on HCT-116 cell line.

viability (~9%) was observed only at 500 µg/ml, while 250 µg/ml resulted in approximately 45% viability, comparable to the positive control. At lower concentrations (100-25 µg/ml), the bark extract exhibited negligible cytotoxicity, with cell viability values close to the untreated control, suggesting a biphasic response. A comparative assessment of IC... € values revealed that *T. tricuspidata* leaf extract was more effective than its bark counterpart in inhibiting HCT-116 cell proliferation. When compared with *Firmiana colorata*, whose bark and leaf extracts exhibited IC_{50} values of 136.3±2.13 µg/ml and 148.4±2.17 µg/ml, respectively, *T. tricuspidata* leaf extract showed comparable cytotoxic potential, whereas the bark extract displayed reduced efficacy. Overall, these findings indicate that the methanolic leaf extract of *T. tricuspidata* possesses appreciable anticancer activity against colon cancer cells and may serve as a promising source of bioactive compounds for further investigation.

The cytotoxic activity observed for the methanolic extracts of *T. tricuspidata* against HCT-116 colon cancer cells is consistent with and extends previous reports on the anticancer potential of medicinal plants rich in phenolics and other secondary metabolites. Concentration-dependent cytotoxic effects have been reported for methanolic extracts of other *Trichosanthes* species against colorectal and breast cancer cell lines, supporting the relevance of solvent polarity and plant part selection in anticancer screening (Babu *et al*., 2024; Prathamanjali *et al*., 2025). Furthermore, the limited activity of the bark extract at lower concentrations suggests a biphasic response, a phenomenon

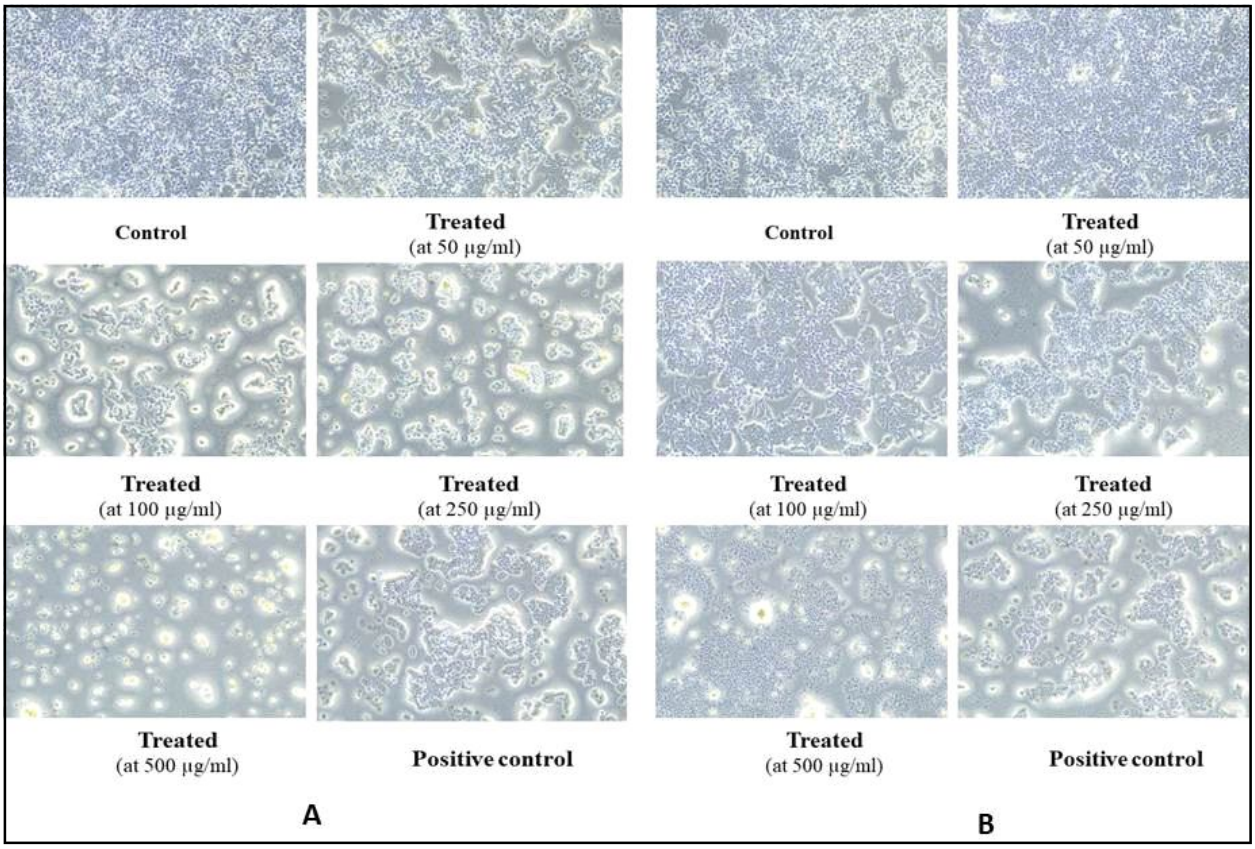

Fig 4. The phase contrast image indicates considerable morphological alterations in HCT-3 cells treated with *T. tricuspidata* methanol extracts: (A) leaf extracts and (B) bark extracts.

previously documented for certain phytochemicals exhibiting hormetic effects at sub-cytotoxic doses. Collectively, these findings corroborate existing literature emphasizing the role of plant-derived compounds as promising, multi-targeted anticancer agents and highlight *T. tricuspidata* leaves as a valuable candidate for further bioactivity-guided fractionation and mechanistic studies.

CONCLUSION

This study provided quantitative evidence that *T. tricuspidata* Lour. exhibited notable antioxidant and anticancer potential. Among the tested extracts, the methanolic bark extract showed the strongest antioxidant activity, with low IC_{50} values in DPPH (35.80±4.04 µg/ml) and FRAP (43.22±0.20 µg/ml) assays, approaching the efficacy of ascorbic acid. Cytotoxicity evaluation against HCT-116 colorectal adenocarcinoma cells revealed concentration-dependent growth inhibition, with the methanolic leaf extract demonstrating higher anticancer efficacy (IC_{50}=129.4±2.11 µg/ml) and over 90% inhibition at 500 µg/ml. GC-MS analysis identified bioactive constituents that may underlie these effects. Overall, *T. tricuspidata*, particularly its methanolic leaf extract, represents a promising source of natural antioxidant and anticancer compounds; however, further *in vivo* validation and mechanistic studies are required.

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