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RESEARCH ARTICLE

Exploration of anti-cancer and apoptosis-inducing potential of three selected ethnomedicinal plants from Northeast India

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Abstract

Traditional Indian medicinal plants have long been valued for their therapeutic potential. Cancer remains a leading global health challenge, with breast and cervical cancers particularly prevalent among women. Although conventional anticancer drugs exist, their adverse effects highlight the need for safer, plant-derived alternatives. This study evaluated the cytotoxic and apoptotic effects of *Zanthoxylum oxyphyllum Edgew*, *Rotheca serrata (L.) Steane & Mabb.* and *Blumea lanceolaria* (Roxb.) Druce on human breast (MCF-7) and cervical (HeLa) cancer cell lines. Cytotoxicity was assessed using the MTT assay and apoptosis was analyzed via Acridine Orange/Ethidium Bromide (AO/Et-Br) dual staining. Results demonstrated a dose-dependent reduction in cell viability across all extracts. *R. serrata* showed the strongest cytotoxicity in MCF-7 cells (IC_{50} =54.53 µg/mL), whereas *B. lanceolaria* was most potent against HeLa cells (IC_{50} =57.14 µg/mL). At the highest concentration tested (100 µg/mL), MCF-7 and HeLa viability decreased to 36.84-45.22 % and 32.21-44.13 %, respectively. AO/Et-Br staining confirmed apoptosis, with a dose-dependent increase in early and late apoptotic cells, corroborating morphological observations of cell shrinkage, rounding and apoptotic body formation. These findings indicate that the investigated plants contain bioactive compounds capable of inducing cytotoxicity and apoptosis in human cancer cells, validating their traditional use. With proper standardization, their leaf extracts hold promise as plant-based anticancer therapeutics.

Keywords: acridine; anticancer; bioactive; cytotoxicity; herbal; traditional

Introduction

Over the past two decades, more than 25 % of medications have been derived from plants, while an additional 25 % have originated from chemically modified natural compounds (1). Cancer remains one of the leading causes of morbidity and mortality worldwide, with breast and cervical cancers ranking among the most prevalent forms in women (2). Conventional treatment strategies including chemotherapy, radiation, surgery and chemically derived drugs are often associated with severe side effects, high costs and limited accessibility (3). This has intensified the global demand for novel and effective anticancer therapeutics with fewer side effects (4).

Medicinal plants are recognized as a vital source of bioactive compounds with cytotoxic and chemo preventive potential. Numerous phytochemicals from edible and medicinal plants have been linked to cancer prevention and therapy (5). Approximately 60 % of current anticancer drugs are derived from natural sources, yet many species with potential anticancer activity remain unexplored (6, 7).

In recent years, ethnomedicinal surveys across South Asia have highlighted the diversity and cultural significance of medicinal plants. Studies from the Palas Valley, Kohistan, Northern Pakistan, have documented the rich ethnomedicinal practices of alpine and subalpine communities (8). In the Kashmir Western Himalaya, ethnobotanical research has revealed the role of medicinal flora in promoting ecological transitions and community-centered health strategies (9). Likewise, investigations in the Neelum Valley and Makra Hills of Azad Jammu & Kashmir have unveiled traditional uses of plants in gynecological disorders, wound healing and cancer-related ailments (10, 11). The nexus between foraging ecology and food security in the Kashmir Himalaya further underscores the importance of wild food plants in sustaining local health systems (12). Collectively, these recent studies demonstrate the persistence and contemporary relevance of indigenous ethnomedicinal knowledge.

Despite these advances, systematic research into the ethnomedicinal resources of Northeast India remains limited, even though the region is recognized as a global biodiversity hotspot. Indigenous communities in Assam and surrounding states rely heavily on herbal remedies, yet the pharmacological validation of many locally used species is still lacking. This gap highlights the need to scientifically evaluate species traditionally used in the region for cancer and related ailments.

Three ethnomedicinally significant species were therefore selected for this study: *Zanthoxylum oxyphyllum* Edgew, *Rotheca*

serrata (L.) Steane & Mabb. and *Blumea lanceolaria* (Roxb.) Druce. These plants are widely used by local populations in Assam and adjoining areas for their therapeutic properties.

Zanthoxylum oxyphyllum Edgew of the Rutaceae family is a scrambling shrub distributed in both tropical and temperate regions. Traditionally, it is used to relieve stomach pain, purify the blood and reduce the risk of leucoderma (13, 14). The bark is applied to treat leg discomfort, varicose veins, ulcers and rheumatism, while the fruits serve as appetizers, anthelmintics and remedies for tumors, gastrointestinal problems and respiratory ailments. Methanolic extracts of the plant have demonstrated analgesic and anti-inflammatory activity (15, 16).

Rotheca serrata (L.) Steane & Mabb. (syn. *Clerodendrum serratum*) of the Lamiaceae family is another important Indian medicinal plant. A documented pharmacological activity of the plant includes anti-inflammatory, antinociceptive, anticancer, hepatoprotective and wound-healing effects (17-20). Additionally, its leaves possess antihypersensitive, antipyretic, antidiabetic and analgesic properties (21, 22).

Blumea lanceolaria (Roxb.) Druce of family Asteraceae, locally known as "Jwglaori," is an evergreen herb distributed widely in Northeast India. Indigenous communities traditionally use its leaves to treat coughs, ulcers, wounds, diarrhea, bronchitis, hemorrhoids and even cancer (23-28).

Given their ethnomedicinal relevance and pharmacological potential, the present study investigates the anticancer activity of methanolic crude extracts of *Z. oxyphyllum*, *R. serrata* and *B. lanceolaria* against human breast cancer (MCF-7) and cervical cancer (HeLa) cell lines.

Materials and Methods

Plant specimen collection and authenticity

The plant materials were collected from Dhupdhara region of Goalpara district in Assam, during the months of September and October, from their native habitats. Herbarium specimens were carefully prepared and dispatched to the Botanical Herbarium of Gauhati University and Bodoland University for taxonomic identification. After verification, the voucher specimens viz., *Zanthoxylum oxyphyllum* Edgew-18924, *Rotheca serrata* (L.) Steane & Mabb-18926 and *Blumea lanceolaria* (Roxb.) Druce-BUBH0000868 were deposited at the Botany Department of Gauhati University and Bodoland University, Assam respectively.

Preparation of the plant extracts

Fresh leaves of *Zanthoxylum oxyphyllum*, *Rotheca serrata* and *Blumea lanceolaria* were collected, shade-dried for 10-15 days and ground into fine powder using a mechanical grinder. For extraction, 50 g of the powdered material was subjected to Soxhlet extraction at 60-65 °C using methanol as solvent. A solvent-to-plant material ratio of 1:10 (w/v) was maintained. Extraction was carried out for 6-7 hr at room temperature with occasional shaking until the solvent became colorless. The crude extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40 °C. The semisolid residue obtained was further dried in a desiccator to remove residual solvent and stored at 4 °C until further use in cytotoxicity assays.

Assessment of the plant extract's cytotoxic effects on cancer cell

Cell strains and culture media

The cancer cell lines were obtained from the National Centre for Cell Science in Pune, India. This study used 2 human cancer cell lines: MCF-7 and HeLa. Stock cells were cultured in MEM supplement contatining 10 % of inactivated Foetal Bovine Serum (FBS), 100 IU/mL of penicillin, 100 µg/mL of streptomycin and 5 µg/mL of amphotericin B in a humidified environment with 5 % of CO_2at 37 °C until confluent. Cell dissociation was performed with a trypsin solution containing 0.2 % of trypsin, 0.02 % of EDTA and 0.05 % of glucose in PBS (Phosphate Buffered Saline). All tests were conducted using 96 microwell plates (Tarsons India Pvt. Ltd., India) and the stock cultures were grown in 25 cm^2 culture flasks.

Cell viability, proliferation and cytotoxicity

Using a yellow water-soluble tetrazolium salt known as the MTT assay (3-(4, 5-dimethyl thiazol-2 yl)-2, 5-diphenyl tetrazolium bromide), cell viability, proliferation and cytotoxicity was evaluated in accordance with procedure outlined (29). For the MTT assay, 100 µL of MEM (minimum essential medium) was added to each well of 96-well plates and cells were seeded at an appropriate density. The plates were then incubated in a CO_2 incubator at 37 °C with 5 % CO_2 in a 95 % humidified atmosphere for 24 hr to ensure proper cell attachment. Following attachment, the cells were treated with different concentrations of the plant samples (5, 10, 25, 50, 75 and 100 µg/mL), while doxorubicin served as a positive control. The plates were then returned to CO_2 incubator for further incubation. 20 µL of MTT solution was applied to each well after 72 hr of incubation to enable the development of formazan crystals, the plates were then incubated for another 2 hr. After carefully aspirating the medium, the plates were incubated for 4 hr to dissolve the formazan crystals in DMSO. After that, absorbance measurements were obtained using a Spectramax M2 Microplate Reader (Molecular Diagnostic, Inc) at 630 nm wavelength. To determine the cytotoxicity activity of the plant extract, the absorbance data were calculated using the formula below:

$$(\%)\ \text{of Cytotoxicity} = \frac{\text{Control - test sample}}{\text{Control}} \times 100$$

Assessment of morphological changes

For assessment of morphological abnormalities of MCF-7 and HeLa cancer cells, the cells were seeded in 96-microwell plates at a density of 2×10^4 cells/well and incubated for 24 hr. Following treatment with the extracts, cell morphology was examined using a phase-contrast inverted microscope (Zeiss, Germany) at 400X magnification after another 24 hr incubation period.

Detection of apoptotic morphological changes using Acridine Orange/Ethidium Bromide (AO/Et-Br) (2:1) staining

The extracts effect on the morphological alterations of HeLa and MCF-7 cells was investigated using acridine orange/ethidium bromide (AO/Et-Br) staining (30). Two DNA-binding dyes, acridine orange (AO) and ethidium bromide (Et-Br), were utilized for the morphological detection of apoptotic and necrotic cells. Both cell lines were plated separately in 96-well plates and left to adhere for 24 hr at 37 °C in 5 % CO_2. Following adherence, the cells were treated with 2 different concentrations: the IC_{50}

concentration and twice the IC_{50} concentration of the plant extracts for 24 hr. The cells were then fixed in 4 % formaldehyde in 1X PBS at room temperature for 15 min after being rinsed with ice-cold 1X PBS (Phosphate Buffered Saline) at pH 7.4 (at a concentration of $1x10^5$ cells/mL). Following that, the cells and dye solution were incubated for 10 min at room temperature in the dark. Doxorubicin was used as a positive control to see apoptotic cells. An inverted fluorescence microscope (Olympus America, Inc., Melville, NY, USA) was used to see the labelled cells.

Statistical data evaluation

Results are presented as mean ± standard deviation (SD). The MTT assay was performed in triplicate to ensure reproducibility. Data analysis was conducted using SPSS software (version 26.0; IBM Corp., Armonk, NY, USA). The Shapiro-Wilk test was used to assess data normality and Levene's test was applied to evaluate homogeneity of variances. The IC_{50} values were determined using linear regression analysis of dose-response curves. For comparison of groups, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was employed. A significance level of $p < 0.05$ was considered statistically significant.

Results

The examination of the cytotoxic activity of the plant extracts utilizing the MTT assay, with doxorubicin as a standard reference, on methanolic extracts (ME) from *Z. oxyphyllum*, *R. serrata* and *B. lanceolaria* in 2 human cancer cell lines, MCF-7 (breast cancer) and HeLa (cervical cancer), demonstrated that the cytotoxicity of each plant extract increased with concentration. The IC_{50} values derived from MCF-7 and HeLa cells are summarized in Table 1,

Table 1. IC_{50} values (µg/mL) of methanolic extracts of *Z. oxyphyllum*, *R. serrata* and *B. lanceolaria* on MCF-7 (breast cancer) and HeLa (cervical cancer) cell lines

Sample	Cytotoxic activity (IC_{50} in µg/mL)	
	MCF-7 (mean ± SD)	HeLa (mean ± SD)
Z. oxyphyllum	77.17 ± 0.24	81.94 ± 0.14
R. serrata	54.68 ± 0.28	85.06 ± 0.08
B. lanceolaria	75.78 ± 0.29	57.34 ± 0.49
Doxorubicin	0.14 ± 0.12	2.38 ± 3.5

Values are expressed as mean ± SD (n = 3). IC_{50} values were calculated using linear regression analysis. Significant differences among groups were assessed using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

showing that *R. serrata* was most potent against MCF-7, while *B. lanceolaria* had the strongest effect on HeLa cells. These quantitative outcomes correspond with the inhibition patterns illustrated in Fig. 1 (MCF-7) & 2 (HeLa), where cell viability decreased progressively with increasing extract concentrations.

The cytotoxic activity of plant extracts elevated as concentrations increased in MCF-7 cell line, suggesting that all 3 plant extracts had improved anticancer activity. An IC_{50} value of less than 100 µg/mL indicated that all 3 samples successfully inhibited the proliferation of MCF-7 cancer cells. The cytotoxicity activity of plant extracts on MCF-7 cells suggested that they considerably decreased the ability of cancer cells to proliferate. This is further supported by the viability percentages shown in Fig. 3, where MCF-7 cells treated at 100 µg/mL exhibited only 36.84-45.22 % survival, consistent with significant cytotoxicity as per ISO:10993-5 standards.

This indicated that MCF-7 cancer cell lines were cytotoxically affected by the sample extracts. Methanolic extract of *R. serrata* exhibited the strongest cytotoxic effect, with a low IC_{50} value of 54.53 µg/mL. Leaf extracts generally exhibited cytotoxic properties, causing a progressive decrease in MCF-7 cell viability at concentrations ranging from 5 to 100 µg/mL. In all samples, cell viability was lowest at the maximum concentration (100 µg/mL) ranging from 36.84 % to 45.22 %. Fig. 1 & 3 depicts the inhibition percentage and cell viability of MCF-7 cells.

The lower IC_{50} values of *Z. oxyphyllum* and *B. lanceolaria* extracts (Table 1) suggested that they were more hazardous to HeLa cells. The corresponding dose-dependent decrease in cell viability is shown in Fig. 2, where HeLa viability declined to 32.21-44.13 % at 100 µg/mL. All extracts were administered at various concentrations that were gradually increased over a 24-hr period. The research shows that *B. lanceolaria*'s methanolic extract effectively suppressed the growth of HeLa cells in a dose-dependent manner. This means that higher concentrations of the extract led to greater inhibition of cell proliferation. *B. lanceolaria* demonstrated significant cytotoxic activity, with a low IC_{50} value of 57.14 µg/mL. Additionally, in the cells treated with doses ranging from 5 to 100 µg/mL different degrees of growth inhibition were seen. Fig. 2 & 4 illustrates the percentage inhibition and cell viability of HeLa cells respectively.

The methanolic extract from *R. serrata* showed cytotoxic activity against HeLa cells, with the lowest viability percentage

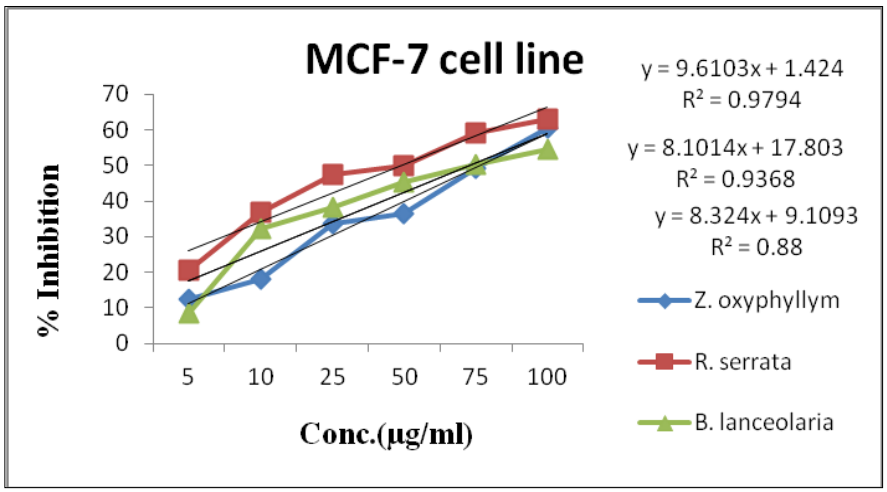

Fig. 1. Dose-dependent inhibition of MCF-7 cell viability after treatment with methanolic extracts of *Z. oxyphyllum*, *R. serrata* and *B. lanceolaria* (24 hr). Values are mean ± SD (n = 3).

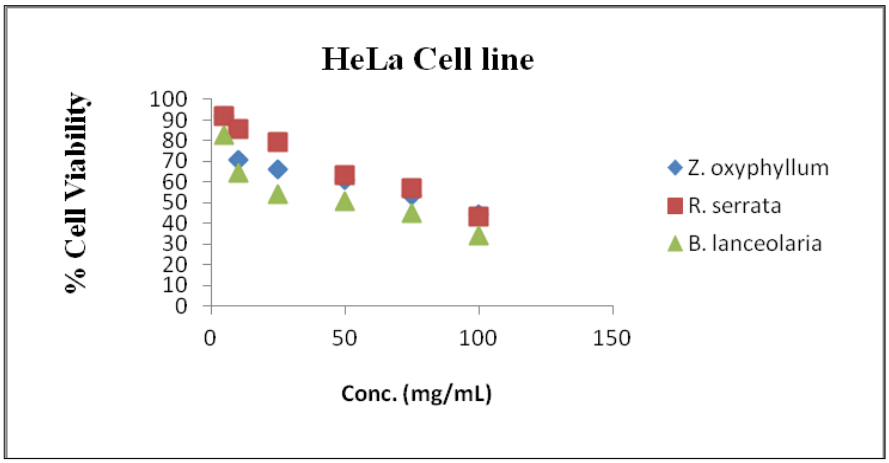

Fig. 2. Percentage cell viability of HeLa cells treated with plant extracts at increasing concentrations.

Data expressed as mean ± SD (n = 3). One-way ANOVA followed by Tukey's post hoc test was applied ($p < 0.05$).

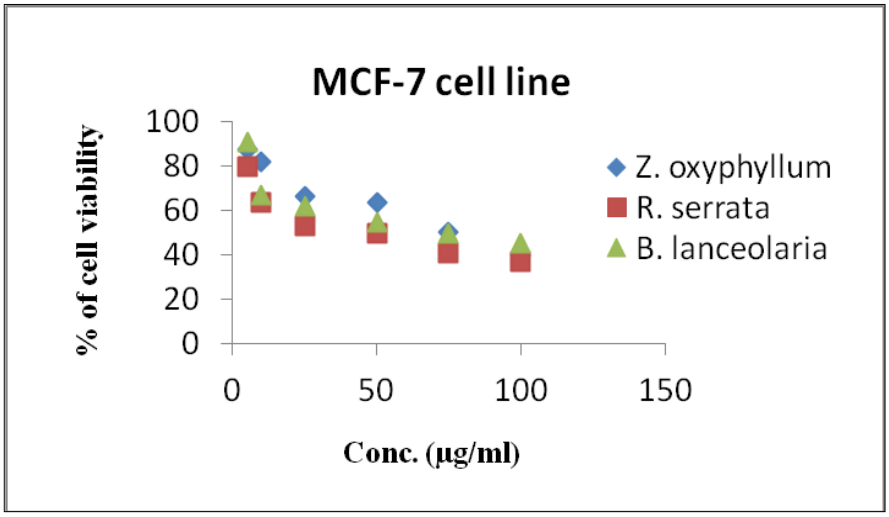

Fig. 3. Percentage cell viability of MCF-7 cells treated with plant extracts at increasing concentrations.

Data expressed as mean ± SD (n = 3). One-way ANOVA followed by Tukey's post hoc test was applied ($p < 0.05$).

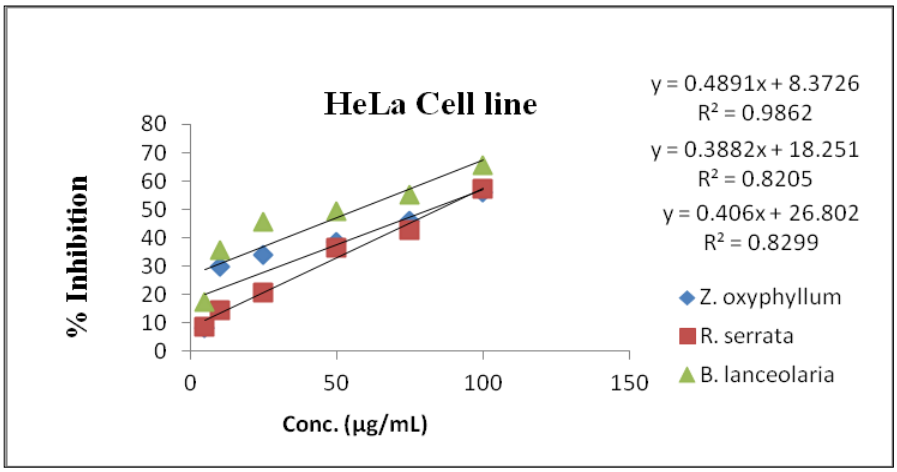

Fig. 4. Dose-dependent inhibition of HeLa cell viability after treatment with methanolic extracts of *Z. oxyphyllum*, *R. serrata* and *B. lanceolaria* (24 hr). Values are mean ± SD (n = 3).

(32.21 % - 44.13 %). At the highest concentration (100 µg/mL), its effect was moderate compared to the other extracts. In contrast, both *Z. oxyphyllum* and *B. lanceolaria* extracts exhibited pronounced cytotoxic effects on HeLa cells (Table 1). These effects were evident through visual observation of cellular morphology which revealed significant abnormalities in the cells after treatment with the extracts (Fig. 5 & 6). Notably, the cytotoxicity increased over time, with the most prominent effects observed at 24 hr, 48 hr and 72 hr.

Observation of morphological changes

MCF-7 and HeLa cell morphological alterations were examined using a phase contrast inverted microscope. As illustrated in Fig. 5 (MCF-7) & 6 (HeLa), untreated control cells maintained their typical morphology, while treated cells exhibited shrinkage, rounding, detachment and apoptotic body formation in a time- and dose-dependent manner. Morphological changes of MCF-7 cells during treatment with plant extracts are shown in Fig. 5. Untreated

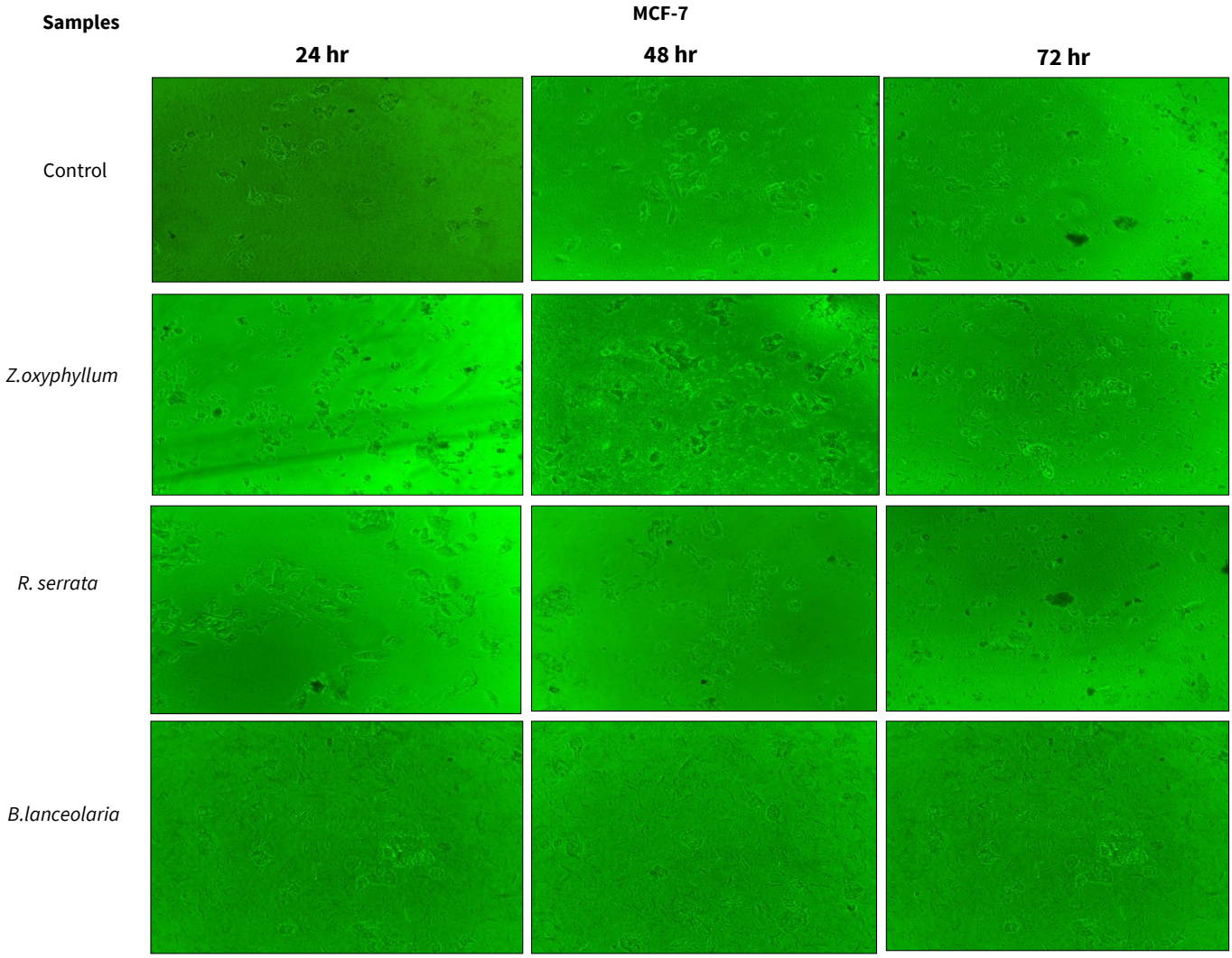

Fig. 5. Morphological changes of MCF-7 cells treated with methanolic plant extracts at different concentrations (24, 48 and 72 hr), showing cell shrinkage, rounding, vacuolization and apoptotic body formation compared to untreated control cells.

control cells retained their normal polygonal or triangular morphology with clear cell boundaries and intact confluency. Upon treatment with methanolic extracts at IC_{50} and $2\times IC_{50}$ concentrations, cells exhibited progressive morphological alterations. This included cell shrinkage, rounding, detachment from the substratum, vacuolization and formation of apoptotic bodies, all characteristic features of apoptosis. At 48-72 hr, an increased number of floating/ suspended cells and fragmented cells were observed, indicating advanced stages of apoptosis and cell death.

Morphological changes of HeLa cells during treatment with plant extracts in Fig. 6. Control cells displayed their typical elongated, spindle-shaped morphology with intact adherence and confluency. After treatment with methanolic extracts at IC_{50} and $2\times IC_{50}$ concentrations, marked morphological alterations were observed, including cell shrinkage, rounding, membrane blebbing and cytoplasmic condensation. With longer exposure (48-72 hr), the number of floating or detached cells increased substantially, accompanied by nuclear fragmentation and apoptotic body formation. These progressive changes are characteristic of apoptosis, confirming that the extracts induce dose- and time-dependent cytotoxic effects in HeLa cells.

AO/Et-Br dual staining method

The AO/Et-Br staining method allows for the calculation of the ratios of early apoptosis, late apoptosis, necrosis and live cells at the IC_{50} and $2\times IC_{50}$ values of the plant extracts. The quantitative outcomes are summarized in Tables 2 & 3, which show the shift from early apoptosis at IC_{50} to late apoptosis at $2\times IC_{50}$, especially in HeLa cells. Representative fluorescence images are provided in Fig. 7 & 8, where viable cells fluoresced green, early apoptotic cells yellow, late apoptotic cells orange/red and necrotic cells bright red. Staining with AO/Et-Br revealed the stages of cell death morphology, exhibiting various colors (Fig. 7 & 8) in both MCF-7 and HeLa cells. Viable or live cells (V) were stained green with intact nuclear structures. Early apoptotic cells (E) appeared yellow, characterized by condensed nuclear structures, cell shrinkage and the production of apoptotic bodies. Late apoptotic cells (L) were stained reddish-orange showing patches of condensed chromatin in the nucleus. Control cells displayed an evenly spaced, circular nucleus in the centre that fluoresced greenish. In contrast, necrotic cells (N) expanded in bulk, unevenly stained and showed uniform red fluorescence at their periphery.

The quantitative results indicated that the extracts from the samples triggered apoptosis in a dose-dependent manner (Table 2 & 3). Higher proportions of early and late apoptotic cells generally indicate higher rates of apoptosis. In MCF-7 cells (Table 2), *R. serrata* induced the highest early apoptosis (48.2 % at IC_{50}), while *Z. oxyphyllum* shifted toward late apoptosis (38.5 % at $2\times IC_{50}$), comparable to the standard drug doxorubicin (45.7 %). *B. lanceolaria* consistently triggered early apoptosis (41.5 %-40.5 %) at both IC_{50} and $2\times IC_{50}$. This suggests that *R. serrata* was more potent in initiating apoptosis at earlier stages, whereas

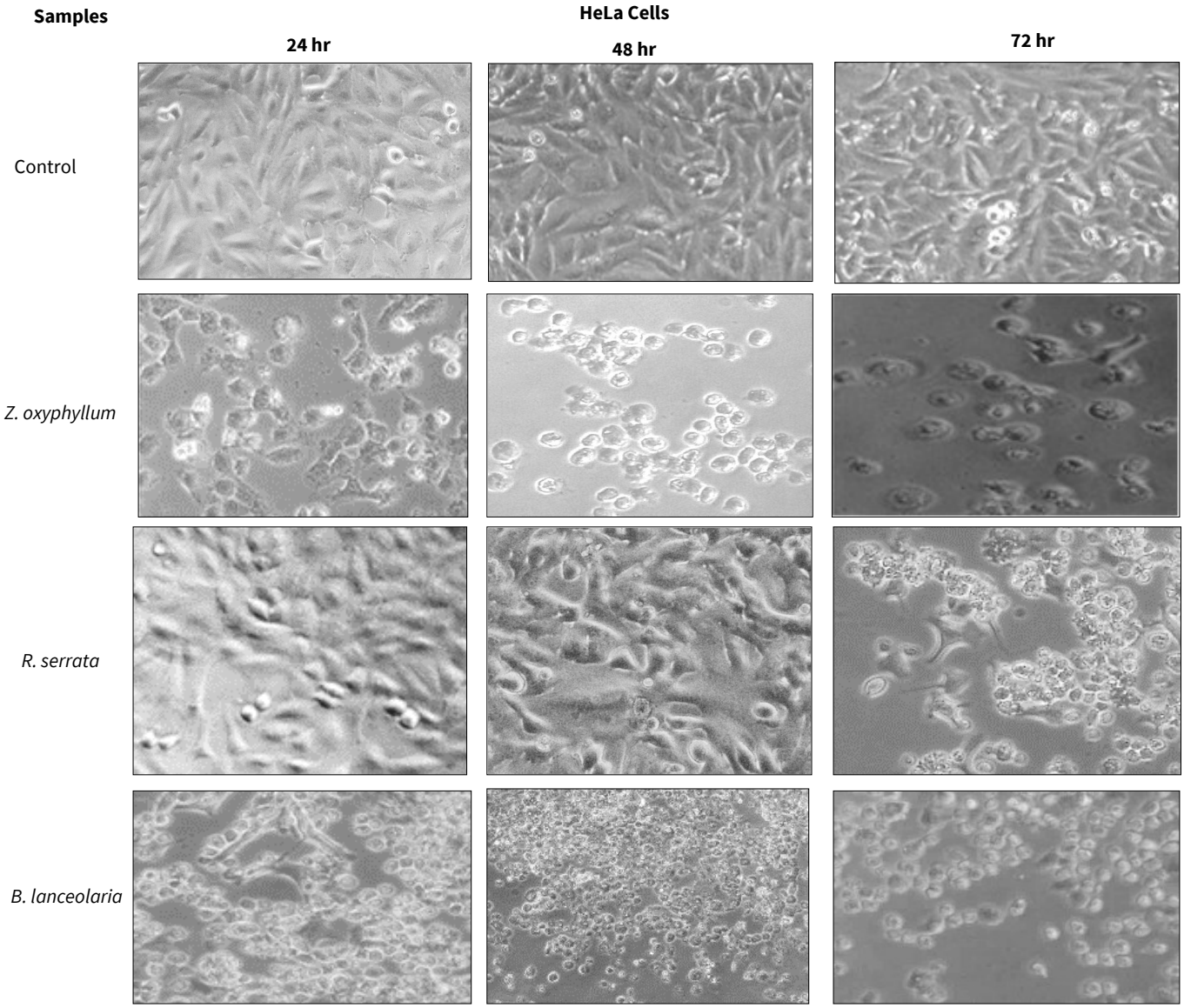

Fig. 6. Morphological changes of HeLa cells treated with methanolic plant extracts at different concentrations (24, 48 and 72 hr), showing cell shrinkage, membrane blebbing and detachment compared to untreated control cells.

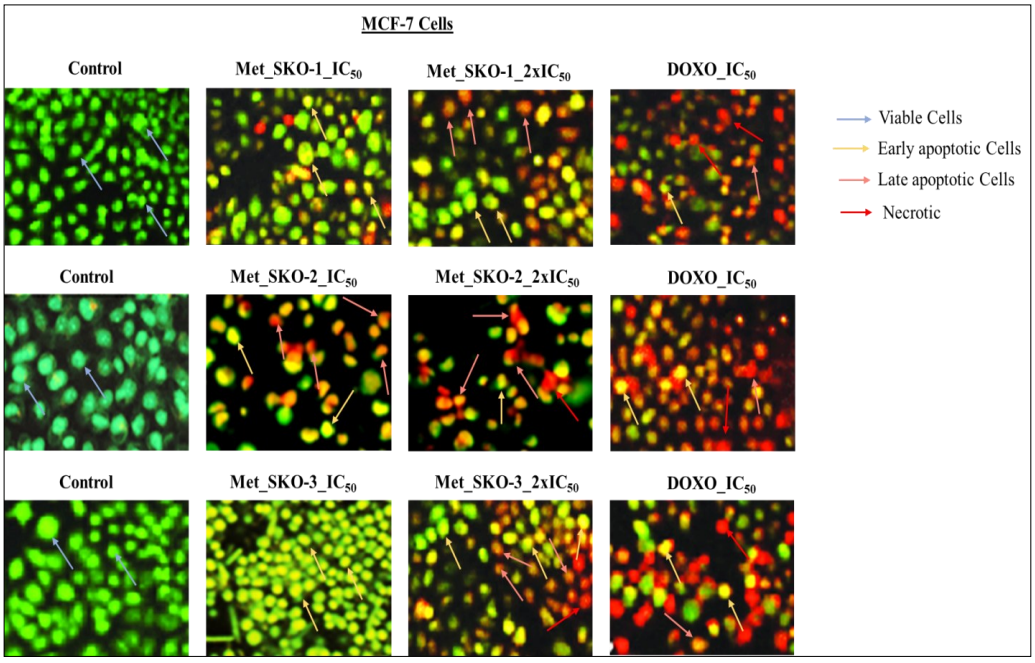

Fig. 7. AO/Et-Br dual staining of MCF-7 cells treated with plant extracts at IC_{50} and $2\times IC_{50}$ concentrations. Viable cells (green), early apoptotic cells (yellow), late apoptotic cells (orange/red) and necrotic cells (red) were observed.

Note: SK0-1 indicates *Z. oxyphyllum*, SK0-2 indicates *R. serrata* & SK0-3 indicates *B. lanceolaria*

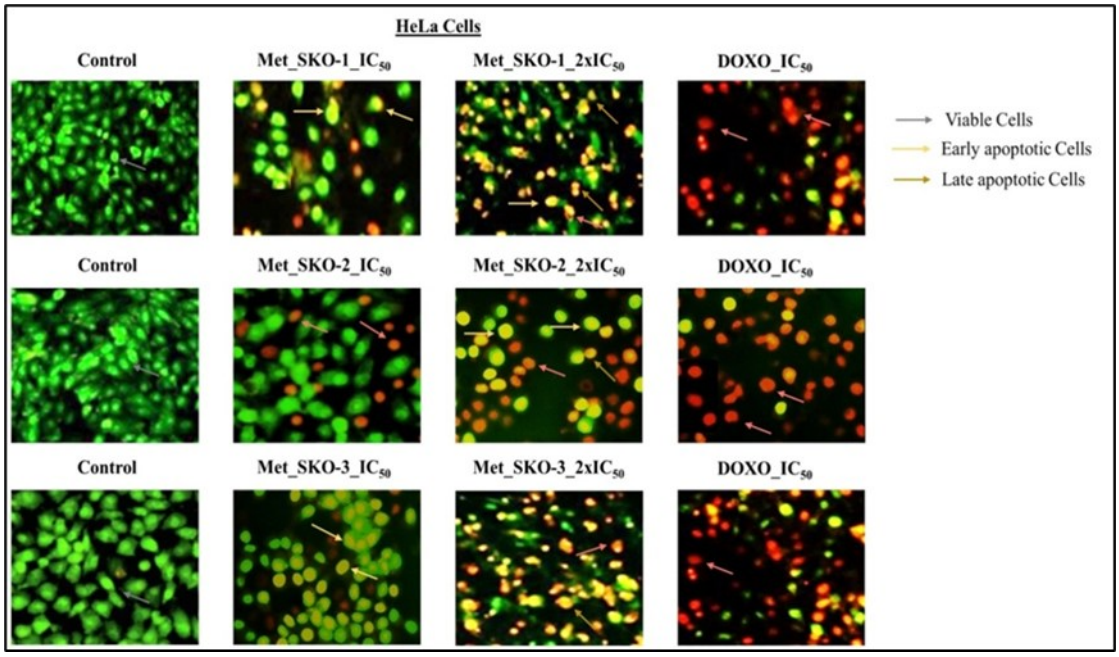

Fig. 8. AO/Et-Br dual staining of HeLa cells treated with plant extracts at IC_{50} and $2\times IC_{50}$ concentrations. Viable cells (green), early apoptotic cells (yellow), late apoptotic cells (orange/red) and necrotic cells (red) were observed.

Note: SK0-1 indicates *Z. oxyphyllum*, SK0-2 indicates *R. serrata* and SK0-3 indicates *B. lanceolaria*

Table 2. Percentage of viable, early apoptotic, late apoptotic and necrotic MCF-7 cells treated with plant extracts at IC_{50} and $2\times IC_{50}$ concentrations compared to doxorubicin (DOXO)

Treatment	% Viable Cells (V)	% Early Apoptotic (E)	% Late Apoptotic (L)	% Necrotic Cells (N)
Control	99.3 ± 8.6	0.1 ± 0.0	0.0 ± 0.0	0.6 ± 0.0
***Z. oxyphyllum* (IC_{50})**	46.3 ± 3.9	39.2 ± 2.4	9.8 ± 1.1	4.7 ± 0.6
***Z. oxyphyllum* ($2\times IC_{50}$)**	21.3 ± 3.6	29.8 ± 3.4	**38.5 ± 4.9**	10.4 ± 0.8
***R. serrata* (IC_{50})**	24.3 ± 2.8	**48.2 ± 5.1**	22.1 ± 2.3	5.4 ± 0.6
***R. serrata* ($2\times IC_{50}$)**	18.5 ± 1.9	26.9 ± 2.7	**47.7 ± 5.3**	6.9 ± 0.7
***B. lanceolaria* (IC_{50})**	47.3 ± 5.6	**41.5 ± 6.2**	6.1 ± 0.8	5.1 ± 0.4
***B. lanceolaria* ($2\times IC_{50}$)**	22.4 ± 2.9	**40.5 ± 4.7**	27.4 ± 3.1	9.7 ± 0.6
DOXO (IC_{50})	16.8 ± 1.2	23.6 ± 2.8	**45.7 ± 5.3**	13.9 ± 1.1

Values are expressed as mean ± SD (n = 3). Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test. Different superscript letters within the same row indicate significant differences ($p < 0.05$).

Table 3. Percentage of viable, early apoptotic, late apoptotic and necrotic HeLa cells treated with plant extracts at IC_{50} and $2\times IC_{50}$ concentrations compared to doxorubicin (DOXO)

Treatment	% Viable Cells (V)	% Early Apoptotic (E)	% Late Apoptotic (L)	% Necrotic Cells (N)
Control	99.1 ± 6.5	0.1 ± 0.01	0.0 ± 0.0	0.8 ± 0.09
***Z. oxyphyllum* (IC_{50})**	43.1 ± 3.1	**34.3 ± 2.6**	16.4 ± 1.2	6.2 ± 0.5
***Z. oxyphyllum* ($2\times IC_{50}$)**	29.4 ± 1.6	26.6 ± 1.3	**34.7 ± 2.7**	9.3 ± 0.8
***R. serrata* (IC_{50})**	56.8 ± 3.9	19.5 ± 1.5	11.3 ± 1.3	12.4 ± 1.8
***R. serrata* ($2\times IC_{50}$)**	41.9 ± 5.7	14.4 ± 1.2	**27.8 ± 1.9**	15.9 ± 1.1
***B. lanceolaria* (IC_{50})**	34.6 ± 2.8	**37.4 ± 2.1**	21.7 ± 1.3	6.3 ± 0.5
***B. lanceolaria* ($2\times IC_{50}$)**	13.5 ± 1.1	22.8 ± 1.6	**54.6 ± 2.7**	9.1 ± 0.8
DOXO (IC_{50})	15.7 ± 1.9	19.1 ± 1.6	**46.5 ± 3.5**	18.7 ± 1.3

Values are expressed as mean ± SD (n = 3). Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test. Different superscript letters within the same row indicate significant differences ($p < 0.05$).

Z. oxyphyllum and doxorubicin promoted a progression toward late apoptosis. In HeLa cells (Table 3), *B. lanceolaria* exhibited the strongest effect, with late apoptosis reaching 54.6 % at $2\times IC_{50}$, exceeding the apoptotic activity of doxorubicin (46.5 %). *Z. oxyphyllum* displayed a dose-dependent switch from early apoptosis (34.3 % at IC_{50}) to late apoptosis (34.7 % at $2\times IC_{50}$), while *R. serrata* showed moderate apoptotic induction with an increase in late apoptosis (27.8 % at $2\times IC_{50}$). Overall, Tables 2 and 3 clarify that all methanolic extracts promoted apoptosis in a concentration-dependent manner, but their activity profiles differed: *R. serrata* was more effective against MCF-7 cells, whereas *B. lanceolaria* showed superior activity against HeLa cells.

Discussion

The MTT assay is a widely employed method for assessing cytotoxic drug therapy and is an analytical tool for chemotherapy (31). In this study, the *in vitro* cytotoxic activity of plant extracts was determined using the MTT test. The assay relies on the ability of living cells to utilize the NADH enzyme to convert yellow MTT into purple formazan. Consequently, cells lose this capacity as they die, which prevents colour formation. The cytotoxic effects of methanolic plant extracts on MCF-7 and HeLa cancer cell lines were investigated. The findings revealed concentration-dependent cytotoxic effects on both cancer cell lines. As the concentration of the plant extract increased, so did cell death,

indicating an escalation in cytotoxicity. Moreover, the cell viability percentage of both MCF-7 and HeLa cells declined with increasing concentrations of the plant extracts.

At the final concentration of 100 µg/mL, the proportion of viable cells decreased following 24 hr treatment with the extracts, as depicted in Fig. 2 & 3. The range of cell viability for MCF-7 and HeLa cells was observed to be between 36.84 % and 45.22 % and 32.21 % to 44.13 %, respectively. According to the ISO:10993-5 standard, cell viability percentages above 80 % are categorized as non-cytotoxic, those between 80 % and 60 % are deemed weak, those between 60 % and 40 % are classified as moderate and those below 40 % indicate significant cytotoxicity (32). Plant extract treatment induced noticeable changes in the morphology of both MCF-7 and HeLa cells. Microscopic examination revealed apoptotic characteristics in both cell types following treatment with the extracts. These morphological changes indicative of cell death was absent in the untreated cells (control group).

The most reliable method for identifying apoptosis is AO/EB staining (33). The morphological and apoptotic mechanisms of the cells were analyzed using the AO/EB staining method, which effectively differentiated between necrotic, early and late apoptotic and surviving cells based on their colour appearance. Utilizing AO/Et-Br labeling, all plant extracts demonstrated a dose-dependent induction of apoptosis in both HeLa and MCF-7 cells. The morphological changes observed in apoptotic cells are frequently utilized as indicators of apoptosis (34). Control (untreated) cells maintained their original shape, as observed via phase contrast microscopy. Conversely, cancer cells treated with the plant extracts for 24, 48 and 72 hr exhibited signs of apoptosis, including autophagy and cell rounding due to shrinkage. After 72 hr of incubation, apoptotic body separation and the development of a reddish-orange hue indicating AO binding to denatured DNA suggested an increase in cell membrane permeability. This study reveals for the first time, that the investigated methanol plants extracts can induce apoptosis in MCF-7 and HeLa human cancer cell lines. Lastly, cells treated with the effective concentration showed clear morphological changes linked to apoptosis.

Previous studies have demonstrated that the methanol extract of *B. lanceolaria* exhibits significant, dose-dependent anticancer activity against the HeLa cell line. As the concentration of the plant extracts increased, the percentage of viable cells dropped. The present results align with previous research (23). *Costus pictus* leaves showed anticancer effects against MCF-7 breast cancer cell lines (35). MCF-7 cells can undergo apoptosis when exposed to the rhizome of *Alpinia galangal* (36). According to a different study, *Thymus vulgaris* and *Thymus serpyllum* methanol extracts were found to have effective doses of 407 µg/mL and 399.407 µg/mL, respectively that caused MCF-7 cells to undergo apoptosis (37). Likewise, the ethanol stem bark extract of *Oroxylum indicum* was reported to do the same in HeLa cancer cells (38). Extracts from *Clinacanthus nutans* have been reported to impede the proliferation of cervical cancer HeLa cells (39).

The phytochemicals in the extract, such as tannins, triterpenes, saponins and polyphenolic substances, are directly linked to its cytotoxic properties (40). Because of their enormous structural diversity, saponins can have anti-tumorigenic effects through a variety of anticancer pathways (41). Natural phenolic compounds have been shown to have lethal effects on human cancer cell lines (42). These polyphenols can eradicate cancer cells by decreasing cell cycle events, triggering apoptosis and altering signaling pathways. Furthermore, polyphenols are noted to regulate the activities of enzymes that stimulate the growth of cancer cells (43). Research has demonstrated that flavonoids impede the proliferation of certain types of cancerous cells (44). Flavonoids from the flavone, flavonol, flavanone and isoflavone families have shown antiproliferative qualities in a range of cancer cell lines, including those from colon, prostate, leukemia, liver, stomach, cervical, pancreatic and breast cancers. It is well-known that certain bioactive compounds can induce apoptosis. Triterpenoids (C30 compounds) have shown great promise in the treatment and prevention of various malignancies (45). The diverse range of habitats that North-East Indian medicinal plants may grow in is one of their beneficial qualities. These plants have naturally occurring terpenoid compounds that can cause cancer cells to undergo apoptosis, as demonstrated through GCMS analysis (46, 47). These compounds have increased anticancer potential, as demonstrated in this study.

The apoptosis-dominant cytotoxicity observed here is consistent with earlier reports of anticancer activity in related taxa. Several *Zanthoxylum* species have shown cytotoxic and pro-apoptotic effects against MCF-7 and other human cancer cell lines, with saponins and alkaloids identified as likely contributors (48, 49). Similar to our findings for *Z. oxyphyllum*, methanolic fractions of *Zanthoxylum* spp. induced apoptosis and dose-dependent reductions in MCF-7 viability. In the case of *Boehmeria nivea*, leaf extracts inhibited MCF-7 proliferation and induced apoptotic changes, paralleling our observation that *B. lanceolaria* strongly induced late apoptosis in HeLa cells (50). Comparable activity has also been described for *R. serrata* and related taxa, where extracts exerted apoptotic and antiproliferative effects in breast cancer cells (51). These parallels reinforce that the apoptotic mechanisms observed in our study are likely mediated by phytoconstituents such as flavonoids, saponins, terpenoids and polyphenols, which are known to trigger apoptosis via mitochondrial pathways, ROS generation, caspase activation and regulation of BCL-2 family proteins (52-54).

Future work should focus on activity-guided fractionation, apoptotic marker assays (e.g., caspase-3/7 activity, PARP cleavage, Bax/Bcl-2 ratio), ROS quantification and testing against normal cell lines to establish selectivity indices. To clarify mechanisms and identify active principles, *in vivo* evaluation would further substantiate the therapeutic potential.

Conclusion

The methanolic extracts of the studied plants have demonstrated the ability to induce apoptosis and exhibit *in vitro* cytotoxic effects on both human breast cancer MCF-7 and cervical cancer HeLa cell lines. This apoptotic activity may be attributed to the presence of naturally occurring phenolic and flavonoid compounds in the extracts. These findings suggest that the investigated plants could be used to develop lead compounds for cancer treatment and other medicinal applications.

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Authors' contributions

SK carried out all laboratory experiments and analysed the data and drafted the initial manuscript. SN assisted in the design of the study, supervised the work and contributed to statistical analysis. Both authors critically revised the manuscript for intellectual content. All authors have read and approved the final version of the manuscript.

Compliance with ethical standards

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