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RESEARCH

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Cytotoxic and immunomodulatory properties of *Tinospora cordifolia*, *Boerhaavia diffusa*, *Berberis aristata*, and *Ocimum basilicum* extracts against HPV-positive cervical cancer cell line

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Abstract

Background Cervical cancer remains a significant public health concern, especially for low-and middle-income countries. This study explores the dual potential of medicinal plant extracts as both anticancer agents and immunomodulators, particularly in HPV-positive cervical cancer, while also addressing the limitations of conventional chemotherapy.

Methods Extracts from *Tinospora cordifolia*, *Boerhaavia diffusa*, *Berberis aristata*, and *Ocimum basilicum* were tested on CaSki and HEK 293 cells alongside cisplatin. Cytotoxicity, genotoxicity, cell migration, HPV DNA inhibition, IFNG secretion, and cell cycle modulation were assessed using established biochemical and immunological assays, including qPCR, ELISA, and flow cytometry.

Results Among the extracts, *B. aristata* demonstrated the strongest anticancer effects on cancer cells while exhibiting minimal impact on normal cells, highlighting its therapeutic potential. It also significantly reduced cell migration and has synergistic affect with cisplatin. *B. diffusa* emerged as the most effective in inhibiting HPV 16 DNA, suggesting its role in viral suppression. Immunomodulatory analysis revealed that *T. cordifolia* and *O. basilicum* significantly enhanced IFNG secretion, indicative of robust immune activation. Each tested extracts induced G0/G1 phase cell cycle arrest, with *T. cordifolia* showing the most pronounced effect.

Conclusions This study highlights the novel therapeutic promise of integrating plant extracts into HPV-positive cervical cancer management. *B. aristata* stands out for its cytotoxicity and anti-migration properties, while *T. cordifolia* offers significant immunomodulatory benefits. These findings pave the way for further research into combining natural products with conventional therapies for safer, more effective cancer treatments.

Keywords Cervical cancer, Medicinal herb, Cytotoxicity, Immunomodulation, Chemoprevention

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Introduction

Cervical cancer ranks as fourth most common cancer amongst all other cancers with an incidence rate of 604,127 new cases and 341,831 deaths globally (Singh et al. [37]). The disease imposes a significant health burden, particularly in lower and middle-income countries where access to screening and prevention is limited. Cervical cancer is predominantly associated with persistent infections caused by high-risk strains of human papillomavirus (HPV), with HPV 16 being the most prevalent and accounting for a substantial proportion of cases. High-risk HPV types are detected in approximately 99% of cervical cancers, making them a necessary factor in the development of this malignancy. Factors such as early onset of sexual activity, multiple sexual partners, and compromised immune responses due to other infections or immunosuppressive states further elevate the risk (Pimple and Mishra [30]). Despite advances in screening technologies such as Pap smears and HPV DNA tests, and the availability of HPV vaccinations, significant challenges persist, especially in under-resourced settings where access to these preventive and diagnostic measures is limited (McGraw [21]). Furthermore, the current treatment modalities, primarily involving platinum-based chemotherapeutics such as cisplatin, face several challenges that limit their effectiveness. Besides their significant nephrotoxic side effects and indiscriminate cytotoxicity, there is an increasing incidence of cancer cell resistance to these drugs, reducing their long-term efficacy (Oun et al. [26]). Moreover, the advanced stages of cervical cancer often show limited response to existing therapies, highlighting a gap in treatment options that could address more severe cases effectively. The high cost and intensive care required for administering these treatments also restrict their accessibility, particularly in regions with limited healthcare infrastructure. Additionally, these treatments can severely impact the quality of life due to their harsh side effects and lack in efficiency to address the underlying HPV infections, which can lead to recurrence. These factors underscore an urgent need for novel therapies that not only provide safer and more effective cancer treatment options but also manage underlying HPV infections,, which contribute to recurrence (Pramesh et al. [32]; Burmeister et al. [4]).

In response to these multifaceted challenges, the exploration of alternative treatments derived from medicinal plants offers a promising avenue for the development of safer and more effective cancer therapies. Medicinal plants, with their diverse phytochemical profiles, have historically been used in various traditional medicine systems across the globe to treat a myriad of ailments, including cancers. These natural compounds often exhibit potent pharmacological activities, such as anti-inflammatory, antioxidant, and direct cytotoxic effects, making them promising candidates for targeting cancer cells while potentially minimizing the side effects associated with conventional chemotherapy (Verma and Singh [39]).

This study specifically investigates the effects of medicinal plant extracts against HPV-positive cervical cancer cells, an area that has not been extensively explored. By examining not only the cytotoxic effects but also the immunomodulatory properties of these extracts, the study aims to address both tumor cell proliferation and immune response modulation, providing a comprehensive therapeutic approach. Additionally, the potential synergistic effects of these plant extracts when combined with cisplatin are evaluated, which could open pathways for complementary therapies that reduce side effects and enhance treatment efficacy. Importantly, this study also explores whether the plant extracts have the capacity to reduce HPV viral load, which could contribute to both tumor suppression and viral control.

Focusing on the potential of specific plants, *Tinospora cordifolia* is known for its immune-modulating effects, suggesting its capabilities to enhance the body's natural defense against cancer. *Boerhaavia diffusa* has demonstrated cytotoxic activity against cancer cells, indicating its potential as a direct anticancer agent. *Berberis aristata* is recognized for its broad-spectrum antimicrobial and antitumor properties, which could contribute to treating cervical cancer and managing associated infections such as HPV (Pai et al. [27]). Lastly, *Ocimum basilicum*, with its rich phenolic content, could provide antioxidant protection as well as inhibit the proliferation of cancer cells. Each of these plants holds a traditional medicinal background, suggesting a reservoir of untapped potential for holistic approaches to cancer treatment that could alleviate some of the limitations faced by current pharmacological therapies. This approach promises a bridge between traditional herbal practices and modern oncological needs, suggesting a fruitful area for further exploration into less toxic, cost-effective, and accessible options for cervical cancer treatment, especially relevant in settings where current therapies are limited or carry significant side effects (Chopra et al. [6]; Pai et al. [27]; Kathirvel and Ravi [14]; Polu et al. [31]).

In this study, an in-depth evaluation of extracts from *T. cordifolia*, *B.diffusa*, *B. aristata*, and *O. basilicum* has been conducted to assess their anticancer properties against HPV-positive cervical cancer cells. Utilizing a variety of in vitro assays, the study examines the cytotoxic, genotoxic, immunomodulatory, and cell cycle effects of the extracts. By focusing on both direct cytotoxicity and immune modulation, as well as exploring their potential synergy with cisplatin, this study offers an

innovative approach to complementary cervical cancer therapy. In the present study, the CaSki (HPV 16) cervical cancer cell line was used for in-vitro experimental analysis. Therefore, the results are suggestive, not conclusive, regarding the therapeutic potential of the extracts.

Methodology

Collection and extraction of phytocomponents from selected plants

The extraction of phytochemical-rich compounds from four different plants, namely *T. cordifolia*, *B. diffusa*, *B. aristate*, and *O. basilicum*, was carried out using powders derived from various parts of these plants. The stems of *T. cordifolia* were selected as they are rich in alkaloids, glycosides, and steroids and known for their potent anticancer properties, making them a valuable part of this study (Puri and Patil [33]). The roots of *B. diffusa* were chosen due to their traditional use and reported antiproliferative effects on cancer cell lines, emphasizing their richness in bioactive compounds (Chopra et al. [6]). The roots of *B. aristata* were picked for its rich composition of berberine, a major alkaloid with well-documented pharmacological activities, including significant anticancer properties (Kumari [18]). The leaves of *O. basilicum* are known to be a rich source of essential oils comprising aromatic compounds such as linalool, estragole, methyl cinnamate, and other significant constituents, including 1,8-cineole, methyl chavicol, eugenol, bergamotene, β-cadinol, limonene, geraniol, and camphor [34]. These compounds are recognized for their therapeutic potential, particularly their anticancer, antioxidant, and anti-inflammatory properties, making basil leaves an important target for exploring bioactive compounds (Perna et al. [29]). *T. cordifolia* stem powder, *B. diffusa* root powder, and *B. aristata* root powder were procured from Lalubhai Vrijlal Gandhi (LVG) store. On the other hand, *O. basilicum* leaves were harvested from a Gujarat University campus, and the formal identification of this plant was performed by Dr. Bharat Maitreya, Professor in the Department of Botany at Gujarat University. A voucher specimen has been deposited in the Gujarat University Herbarium (Accession No. GU/BOT/L/O27). After collection, the leaves were meticulously washed, dried to eliminate moisture, and ground to a fine powder. Subsequently, the prepared powders were subjected to a Soxhlet extraction process to obtain phytochemically rich extracts. Briefly, 20 g of each plant powder was packed into a Whatman filter paper no. 1 thimble, mixed with a 70:30 (v/v) ethanol-water mixture (200 ml) in a round-bottom flask. The extraction process continued for 18 h on the Soxhlet assembly, until the solvent in the apparatus became colourless or nearly colourless, indicating completion of the extraction. The liquid extracts were then concentrated by evaporating the solvent in a hot air oven. This drying process continued until a constant weight was achieved, confirming that all residual solvent had been removed. The resulting extracts were stored at 4 °C for further experimental analysis.

Phytochemical screening of plant extracts

Phytochemical screening was performed to identify the active constituents within the hydroalcoholic extracts of the plants. Qualitative assays were conducted to detect the presence of flavonoids, tannins, phenolic compounds, alkaloids, steroids, di-terpenoids, carbohydrates, and proteins. These tests utilized the hydroalcoholic extracts from *T. cordifolia*, *B. diffusa*, *B. aristata*, and *O. basilicum*, labelled respectively as TCE, BDE, BAE, and OBE. The screening protocol was based on the methods outlined in Harborne [10] ensuring a thorough and standardized approach to the qualitative analysis of the phytochemicals.

Sourcing and culturing of cell lines

The CaSki cell line, used in this study, carries genetic material of HPV 16, which is a high-risk strain associated with cervical cancer, along with the HEK 293 cell line, derived from human embryonic kidney tissue, were both obtained from the National Centre for Cell Science (NCCS) in Pune, India. These cells were then cultured in Roswell Park Memorial Institute (RPMI-1640; Cat#11875093, Gibco) and Dulbecco's modified Eagle's medium (DMEM; Cat#SH30021.01, HyClone) media, respectively. The growth media were enriched with 10% Fetal Bovine Serum – FBS (Cat#26140079, Gibco) and a combination of penicillin and streptomycin antibiotics (Cat#15140122, Gibco). The additional supplements required for cell growth were added in accordance with the specifications outlined in the provided cell line template. The cell lines were maintained in a controlled environment in a humidified incubator at temperature of 37 °C and an atmosphere containing 5% CO_2 to facilitate proper growth and maintenance.

Cell proliferation and combination index analysis by MTT Assay

The cytotoxic effects of TCE, BDE, BAE, OBE and cisplatin (CIS), both individually and in combination, were assessed using the MTT assay. The assay protocol was adapted from Mosmann [23]. For each plant extract, 1 mg of the extract was initially dissolved in 100 µL of dimethyl sulfoxide (DMSO). Following this, 900 µL of cell culture media was added to obtain a 1 mg/mL stock solution. This stock solution was subsequently diluted in culture media to achieve the working concentrations used in the cytotoxicity assays, which ranged from 10 µg/

mL to 1000 µg/mL. For cisplatin, a 1 mg/mL solution was received and used to prepare a 500 µM stock solution by dilution in phosphate-buffered saline (PBS). From this stock, the working concentrations for cisplatin were further diluted in media, yielding a final range of 1 µM to 500 µM for cytotoxicity assessments.

Cell seeding in 96-well plates was performed at densities aimed to achieve 70–80% confluence: 12×10^3 cells/well for CaSki and 20×10^3 cells/well for HEK 293, under standard culture conditions. Following a 24-hour incubation, the cells were treated with the prepared plant extract and cisplatin concentrations, as well as combinations of 100 µM (30.03 µg/mL) cisplatin with varying concentrations of the plant extracts.

After 24 h of treatment, 10 µL of MTT solution (5 mg/mL) was added to each well, and incubation continued for three hours. Formazan crystals formed were dissolved by removing the supernatant and adding 100 µL of DMSO. The absorbance was measured at 570 nm using a microplate reader (Epoch BioteK, USA). Cell viability was calculated using the following equation.

$$Viability\ (\%) = \left(\frac{A570,\ treated}{A570,\ control}\right) \times\ 100$$

The combination index (CI) for the effects of plant extract and cisplatin combinations was analyzed using Compusyn software version 1.0. Each experiment was conducted at least three times to ensure reproducibility and reliability of the results.

Effect of plant extracts on cell cycle progression

The impact of TCE, BDE, BAE and OBE each at a concentration of IC_{50} values of the respective plants, on the cell cycle of CaSki cells was investigated. A commercially available cell cycle analysis kit (ab287852; Abcam) was employed according to the manufacturer's instructions. Initially, 3×10^5 CaSki cells were seeded in a 6-well plate and incubated for 24 h at 37 °C and 5% CO_2 incubator. As per the protocol, the cells were then incubated for an additional 24 h in a medium containing 0.1% FBS. Following, the cells were exposed to each plant extract in a medium supplemented with 10% FBS for another 24 h. Control cells were maintained in a medium containing 10% FBS without any plant extract. After the treatment period, cells were harvested and washed with cold phosphate-buffered saline (PBS). They were then fixed in 70% ethanol and stained using the provided staining solution in the kit. Analysis of cell cycle distribution was performed using a MacsQuant 10 analyzer (Miltenyi Biotech), with the laser set to 488 nm. The results were quantified and are presented as the percentage of cells in each phase of the cell cycle, providing insights into the effects of the plant extracts on cell cycle dynamics.

Comet assay for genotoxicity evaluation

The Comet assay, also known as single cell gel electrophoresis, was utilized to assess the level of DNA damage in individual HEK293 cells. Protocol was followed from (Nandhakumar et al. [24]). Briefly, cells were plated in 6-well plates and incubated at 37 °C in a 5% CO_2 atmosphere for 24 h to allow cell morphology stabilization. After this period, the cells were treated with the IC_{50} concentrations of the plant extracts TCE, BDE, BAE, OBE, and the chemotherapeutic agent cisplatin (30.03 µg/ml) for an additional 24 h under the same conditions. Control cells were incubated with only the media. Following treatment, cells were harvested using trypsinization and prepared for the assay. They were embedded in 0.5% low melting point agarose over a base layer of 0.75% normal melting agarose on microscope slides, which were subsequently chilled at 4 °C. Cell lysis was facilitated by immersing the slides in an alkaline lysis solution (pH 10.0) containing sodium chloride, disodium EDTA, Tris, Sodium Dodecyl Sulfate, Triton-X, and DMSO, maintained at 4 °C for four hours. The slides were then placed in a pre-cooled electrophoresis buffer for 30 min before undergoing electrophoresis at 300 mA for 30 min, positioned perpendicular to the electrodes. After electrophoresis, the slides were neutralized in a Tris buffer (pH 7.4) for five minutes and rinsed several times with double-distilled water.

For visualization, the DNA was stained with 50 µL of ethidium bromide (2 µg/mL), covered with a clean coverslip, and excess stain was carefully removed. Observations were made using a fluorescent microscope (Zeiss, Germany) equipped with a Rhodamine excitation filter (515–560 nm wavelength) at 20X magnification, allowing for the assessment of DNA damage both visually and quantitatively.

Assessment of migration ability of CaSki cells

The wound healing assay was employed to evaluate the migration potential of CaSki cells, providing insight into the effects of the plant extracts on the invasive behaviour of cancer cells. This assay is particularly relevant in assessing how changes in cell viability and DNA integrity, induced by the extracts, might influence cell movement and proliferation. CaSki cells were seeded at a density of 1×10^5 cells/well in a 12-well plate and incubated at 37 °C in an atmosphere of 5% CO_2. After 24 h, when cells reached approximately 70–80% confluence, a sterile 200 µL micropipette tip was used to create a precise scratch down the center of each well. Loose cells were removed by gently washing the wells with PBS. Following this, the cells were exposed to the IC_{50} concentrations of TCE, BDE, BAE and OBE for 24 h. Control cells were treated

with the corresponding media without extracts. Photographic documentation of the scratch was conducted immediately after making the scratch and again at the end of the 24-hour treatment period using a camera attached to an inverted microscope (Zeiss, Germany). The change in the area of the scratch, indicating cell migration to close the wound, was quantitatively analyzed using Image J software. This analysis allowed for the measurement of the rate and extent of cell migration, providing valuable data on how each extract influences the migration capabilities of HPV-positive cervical cancer cells.

Chemo preventive assessment of plant extracts on human papilloma virus (HPV)

This evaluation is essential for understanding how changes in cell viability, cell cycle, and DNA integrity might impact HPV, which is central to cervical cancer's pathology. CaSki cells were seeded at a density of 3×10^5 cells per well in a 6-well plate and incubated under standard conditions (37 °C, 5% CO_2) for 24 h. Following this incubation, the cells were treated for 24 h with IC_{50} concentrations of the extracts, prepared in RPMI-1640 complete medium. Control cells were left untreated to serve as a baseline for comparison. Post-treatment, the cells were detached using trypsinization and subsequently processed for HPV detection. The detection and quantification of HPV DNA were conducted using the RT-PCR method, employing kits and protocols as described by previous study (Harlé et al. [11]). The analysis differentiated between the total HPV DNA present, regardless of its activity state, and provided a quantitative measure of the variance in HPV DNA levels between the control and the treated cells.

Assessment of immunomodulatory properties

IFNG quantification assay

Following the treatment of CaSki cells with plant extracts for chemoprevention assessment, the remaining cell culture supernatant was collected to analyze Interferon gamma (IFNG) levels. IFNG quantification was performed using an ELISA based on the Quantikine ELISA kit (cat#DIF50C, R&D Systems, USA) following the manufacturer's detailed protocol. This assay allowed for the measurement of IFNG levels in the supernatant, providing an indicator of the immunomodulatory effect exerted by the plant extracts.

CD markers quantification assay

To further evaluate the immunomodulatory effects of the plant, Peripheral blood mononuclear cells (PBMCs) were isolated from umbilical cord blood obtained from the Department of Obstetrics and Gynecology at Smt. NHL Municipal Medical College (MMC), Ellis Bridge, Ahmedabad, Gujarat, India. This study was reviewed and approved by the Institutional Ethics Committee of Gujarat University, Ahmedabad, Gujarat, India (Approval Number: GU/IEC/11/2019). The umbilical cord blood samples used in this study were leftover materials that would otherwise be discarded and the need for consent was waived by the Institutional Ethics Committee. The PBMCs were isolated using Ficoll density gradient centrifugation as described by previously reported study (Torabi et al. [38]). After isolation, the mononuclear cells were cultured in RPMI-1640 medium enriched with 1.5 g/L sodium bicarbonate, 1 mM sodium pyruvate, 1X antibiotics (penicillin and streptomycin), and HEPES. This was followed by a 24-hour incubation period to allow for monocyte adherence. Subsequently, the medium was refreshed, and the non-adherent cells were cultured and stimulated with phytohaemagglutinin (PHA) to induce lymphocyte proliferation over several days as per the protocol described in previous study (Zaki Molvi [45]). After this proliferation phase, the cells were transferred to TexMACS™ GMP Medium (Cat#130-097-196, Miltenyi Biotec) to promote T-cell expansion as per manufacturer guidelines. Once confluent T cell colonies were established, they were exposed to the respective IC_{50} concentrations of the plant extracts for 24 h in a 6-well plate.

The impact of these extracts on various immune cell subsets, including lymphocytes, T-cells, B-cells, and NK cells, was analyzed using flow cytometry. Specific markers were utilized to identify each immune cell subset during flow cytometry analysis. CD3 + markers were used to detect the general T cell population, while CD4 + CD3 + markers identified helper T cells, a critical subset for modulating immune responses. CD19 + markers represented B cells, which are essential for humoral immunity and antibody production. Additionally, CD56 + markers were employed to detect NK cells. This analysis was performed on a MacsQuant 10 Analyzer (Miltenyi Biotec), allowing for the detailed characterization of changes in the immune cell profiles in response to the plant extract treatments.

Statistical analysis

Statistical analyses were performed using GraphPad PRISM version 8.0.1. The data obtained from each experiment, conducted in triplicate, were analysed using Student's t-tests or one-way ANOVA where appropriate. Results are presented as mean ± standard error of the mean (SEM) derived from three independent experiments. A P value of less than 0.05 was considered statistically significant, indicating that the observed differences were unlikely to be due to chance.

Result and discussion

Qualitative analysis of phytocompounds in plant extracts

The qualitative screening of phytocompounds from the extracts revealed a diverse range of bioactive compounds. The presence of flavonoids, tannins, phenolic compounds, alkaloids, steroids, di-terpenoids, carbohydrates, and proteins were assessed and quantified, as summarized in Table S1. TCE exhibited high levels of steroids and diterpenoids, compounds known for their anti-inflammatory and anticancer properties. This finding is consistent with previous studies that have identified similar phytoconstituents in *T. cordifolia* and linked them to its therapeutic effects (Kulkarni et al. [16]). On the other hand, BAE and BDE demonstrated moderate to high levels of alkaloids and steroids. Alkaloids, such as berberine found in *B. aristata*, have been extensively studied for their antimicrobial and anticancer activities (Palmieri et al. [28]). Similarly, the presence of these compounds in BDE supports its traditional use in treating various ailments (Das et al. [7]). OBE showed a balanced profile with moderate presence across most tested phytochemical groups. This comprehensive phytochemical composition underpins its versatile application in traditional medicine for a variety of health conditions.

While this qualitative analysis highlights the presence of bioactive groups, the specific concentration and identity of individual compounds may vary due to factors such as geographical origin and environmental conditions. Advanced analytical methods like HPLC, LC-MS, or GC-MS could provide a more precise identification and quantification of key bioactive constituents. This detailed profiling would allow for correlation of specific compounds with observed biological activities, enhancing mechanistic insights and ensuring consistency across extract batches.

Cytotoxic effects of plant extracts and cisplatin on CaSki and HEK 293 cell lines

The study focused on the in vitro cytotoxic effects of TCE, BDE, BAE, OBE, alongside cisplatin (CIS) on two cell lines: CaSki, a cervical cancer cell line, and HEK 293, a normal cell line. IC_{50} values, indicating the concentration required to reduce cell viability by 50%, are summarized in Table S2 for both cell lines.

Dose-response curves for cytotoxicity evaluation of the selected plant extracts and cisplatin on CaSki and HEK 293 cell lines are shown in Fig. 1a and b respectively. Both figures demonstrate a dose-dependent

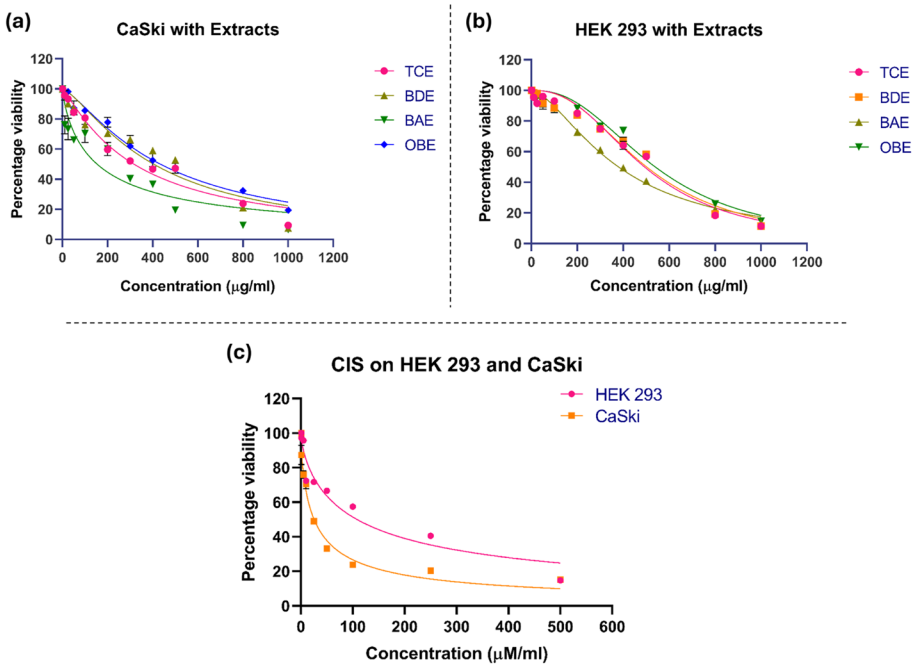

Fig. 1 Dose-Response Curves for Cytotoxicity Evaluation of Plant Extracts and Cisplatin on CaSki and HEK 293 Cell Lines (**a**) Dose-response curve showing the percentage of cell viability for CaSki cells treated with TCE, BDE, BAE, and OBE at varying concentrations. **b** Dose-response curve for HEK 293 cells treated with the same plant extracts, highlighting their relative effects on non-cancerous cells. **c** Comparative dose-response curve for cisplatin (CIS) on CaSki and HEK 293 cell lines, illustrating its high cytotoxicity towards cancerous cells (CaSki) with significant effects on non-cancerous cells (HEK 293). Each data point represents the mean percentage viability from three independent experiments, with error bars indicating the standard error of the mean (SEM). [*Tinospora cordifolia* extract (TCE), *Boerhaavia diffusa* extract (BDE), *Berberis aristata* extract (BAE), and *Ocimum basilicum* extract (OBE)]

decrease in cell viability with increasing concentrations of each plant extract, indicating their potential cytotoxic effects. On the other hand, Fig. 1c represents the comparative cytotoxic effect of cisplatin on both CaSki and HEK 293 cell lines, showcasing its universally potent cytotoxicity, reflected by lower IC_{50} values across both cell types. It is noticeable that Cisplatin, while effective, is known for its broad cytotoxicity and associated side effects such as nephrotoxicity often limit its utility (Hallajzadeh et al. [9]).

BAE exhibited cytotoxic effects on cancer cells, underscoring its potential as a complementary therapeutic option. BAE's cytotoxic effects may be partially attributed to the presence of berberine, a major alkaloid of the plant. Studies have shown that berberine induces apoptosis and inhibits cell proliferation in cancer cells through mechanisms such as reactive oxygen species (ROS) generation and DNA damage (Xie et al. [44]). This suggests that berberine, along with other bioactive components in BAE, contributes to the extract's observed cytotoxicity, potentially via synergistic interactions among its phytochemicals. In contrast, OBE displayed the least cytotoxicity among the extracts on both cell lines, possibly due to the nature of its primary constituents, which might exert milder effects on cellular viability. Essential oils from *O. basilicum* have shown cytotoxicity in previous studies, but the moderate effect observed in this study may reflect differences in extract preparation or constituent concentrations (Kathirvel and Ravi [14]). TCE and BDE displayed intermediate cytotoxicity, suggesting a balance between their anticancer and mild effects on normal cells, possibly due to their diverse phytochemical profiles, including flavonoids and alkaloids.

The comparison of these extracts with cisplatin underscores their potential as less toxic alternatives for complementary cancer therapy. While cisplatin's universal cytotoxicity highlights its efficacy, its adverse effects remain a significant barrier to patient-friendly treatment (Sánchez-González et al. [35]). Conversely, the mild impact of plant extracts on HEK 293 cells suggests their potential to minimize the collateral damage typical of standard chemotherapy. The observed cytotoxic effects of plant extracts such as TCE and BAE align with previous research demonstrating the potential of natural products, such as those from *T. articulata*, to target cancer cells effectively while reducing toxicity to normal cells, underscoring the potential of phytochemicals as anticancer agents (Calderón-Montaño et al. [5]).

The results from this study indicate that BAE demonstrates a promising cytotoxic effect on cancer cells with a relatively mild impact on normal cells in vitro. Though, further in vivo studies and clinical trials are necessary to confirm its safety and efficacy before considering its incorporation into cancer treatment regimens.

Evaluation of Combination Index for Cisplatin and Plant extracts on CaSki cells

The interaction between cisplatin (CIS) and various plant extracts (TCE, BDE, BAE, OBE) on CaSki cells was rigorously evaluated using the combination index (CI), a measure that helps discern whether drug interactions are synergistic, additive, or antagonistic. Figure S1 visually compares the viability of both CaSki and HEK 293 cells following the treatment with various concentrations of these medicinal herb combinations with CIS and CIS alone. The observed cytotoxicity of the combination study indicates potential therapeutic benefits in targeting cancerous CaSki cells, though further studies are required to minimize the impact on normal HEK 293 cells.

Figure 2 showcases normalized isobolograms plotting CI values against varying ratios of the plant extracts. Here, the diagonal line from (0,1) to (1,0), known as the line of additivity, is a critical reference; data points below this line signify synergy, points on the line suggest additive effects, and those above indicate antagonism. From the analysis depicted in Fig. 2, most combinations of CIS and TCE align near or below the line of additivity, indicating these combinations are generally additive or mildly synergistic, particularly at higher TCE concentrations. For CIS combined with BDE, the points scatter about the additivity line, some dipping below, suggesting a predominantly additive interaction with moments of synergy, especially at lower BDE concentrations. Intriguingly, CIS and BAE interactions mostly plot below the line, signaling robust synergy across a spectrum of ratios and identifying this pair as particularly efficacious. However, CIS combined with OBE tends to sit near or slightly above the line, pointing to additive or marginally antagonistic effects, especially at higher concentrations of OBE. Moreover, Table 1 quantitatively illustrates the interaction between various concentrations of each plant extract and a constant dose of CIS, offering insights into the potential synergistic or antagonistic effects. These botanical components may exhibit alongside standard chemotherapy treatments. Table 1 demonstrates a nuanced impact of combining specific plant extracts with cisplatin, as evidenced by the CI values across different concentrations. The strong synergy observed particularly with BAE suggests potential for reducing CIS dosage, thereby possibly reducing associated toxic side effects while maintaining therapeutic efficacy [43]. This detailed understanding of drug interactions underscores the potential of integrating plant

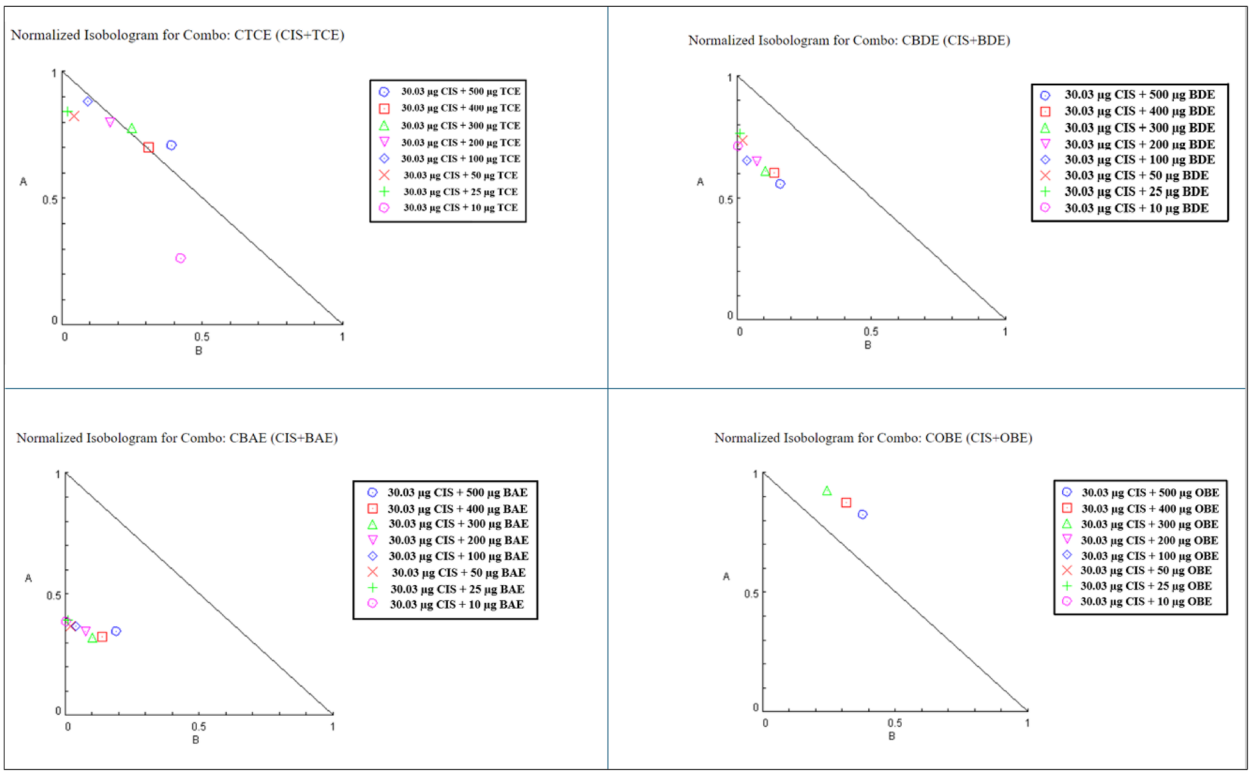

Fig. 2 Normalized Isobolograms for Cisplatin and Plant Extract Combinations. This figure displays the interaction effects between cisplatin (CIS) and plant extracts TCE, BDE, BAE, and OBE on CaSki cells, represented in four panels. Each panel shows the combination index (CI) results plotted against the fraction affected for different concentrations of plant extracts combined with a constant dose of CIS. Points below the diagonal line indicate synergistic effects, points on the line indicate additive effects, and points above the line indicate antagonistic interactions [*Tinospora cordifolia* extract (TCE), *Boerhaavia diffusa* extract (BDE), *Berberis aristata* extract (BAE), and *Ocimum basilicum* extract (OBE)]

Table 1 Combination index (CI) for cisplatin with various plant extracts [*Tinospora cordifolia* extract (TCE), *Boerhaavia diffusa* extract (BDE), *Berberis aristata* extract (BAE), and *Ocimum basilicum* extract (OBE)] across different concentrations

Dose CIS (µg/ml)	Dose plant extracts (µg/ml)	CI of TCE	CI of BDE	CI of BAE	CI of OBE
30.03	500	1.10	0.72	0.54	1.21
30.03	400	1.01	0.74	0.46	1.19
30.03	300	1.03	0.72	0.43	1.17
30.03	200	0.97	0.72	0.42	1.20
30.03	100	0.97	0.69	0.41	1.28
30.03	50	0.87	0.76	0.39	1.47
30.03	25	0.86	0.78	0.40	1.60
30.03	10	0.69	0.72	0.39	1.74

extracts into conventional chemotherapeutic regimens to enhance efficacy and reduce adverse effects, paving the way for more refined cancer treatment strategies. Integrating traditional medicine with conventional chemotherapy could be a viable strategy for enhancing the effectiveness of cancer treatments (Wang et al. [42]). The combination of CIS and plant extracts demonstrated enhanced cytotoxic effects on CaSki cells compared to HEK 293 cells. The combination treatments reduced viability in cancer cells, the differences in cytotoxic effects between CaSki and HEK 293 cells suggest potential therapeutic benefits but require further investigation for clinical relevance.

Evaluation of the cell cycle analysis

The cell cycle analysis of CaSki cells treated with various plant extracts revealed significant alterations in cell cycle distribution, indicative of a cell cycle arrest. Flow cytometric analysis demonstrated that the control group (untreated) exhibited a typical cell cycle distribution across the G0/G1, S, and G2/M phases. However, treatment with TCE and other extracts such as BDE, BAE, and OBE resulted in notable changes.

Figure 3a and b illustrate these effects, showing an increased percentage of cells in the G0/G1 phase and a concomitant decrease in the S phase population upon treatment with the plant extracts. This pattern suggests that the extracts induce cell cycle arrest at the G0/G1 checkpoint, thereby inhibiting progression to the DNA synthesis (S) phase and cell division (G2/M) phase. Specifically, TCE treatment significantly elevated the G0/G1 phase cell population, suggesting a robust induction of G0/G1 phase arrest. This was accompanied by a marked reduction in the S phase, reinforcing the anti-proliferative impact of TCE.

In contrast, the effects of BDE, BAE, and OBE, while notable, were less pronounced compared to TCE. Interestingly, OBE-treated cells displayed an increased proportion in the G2/M phase, potentially indicating a secondary checkpoint arrest or the accumulation of cells unable to complete mitosis. This dual checkpoint modulation could reflect a distinct mechanism of action for OBE, warranting further investigation.

These observations align with existing research on the anticancer properties of TCE, which has demonstrated similar effects in other cancer cell lines. For instance, *T. cordifolia* has been shown to induce G0/G1 phase arrest in glioma cells, reducing proliferation in a

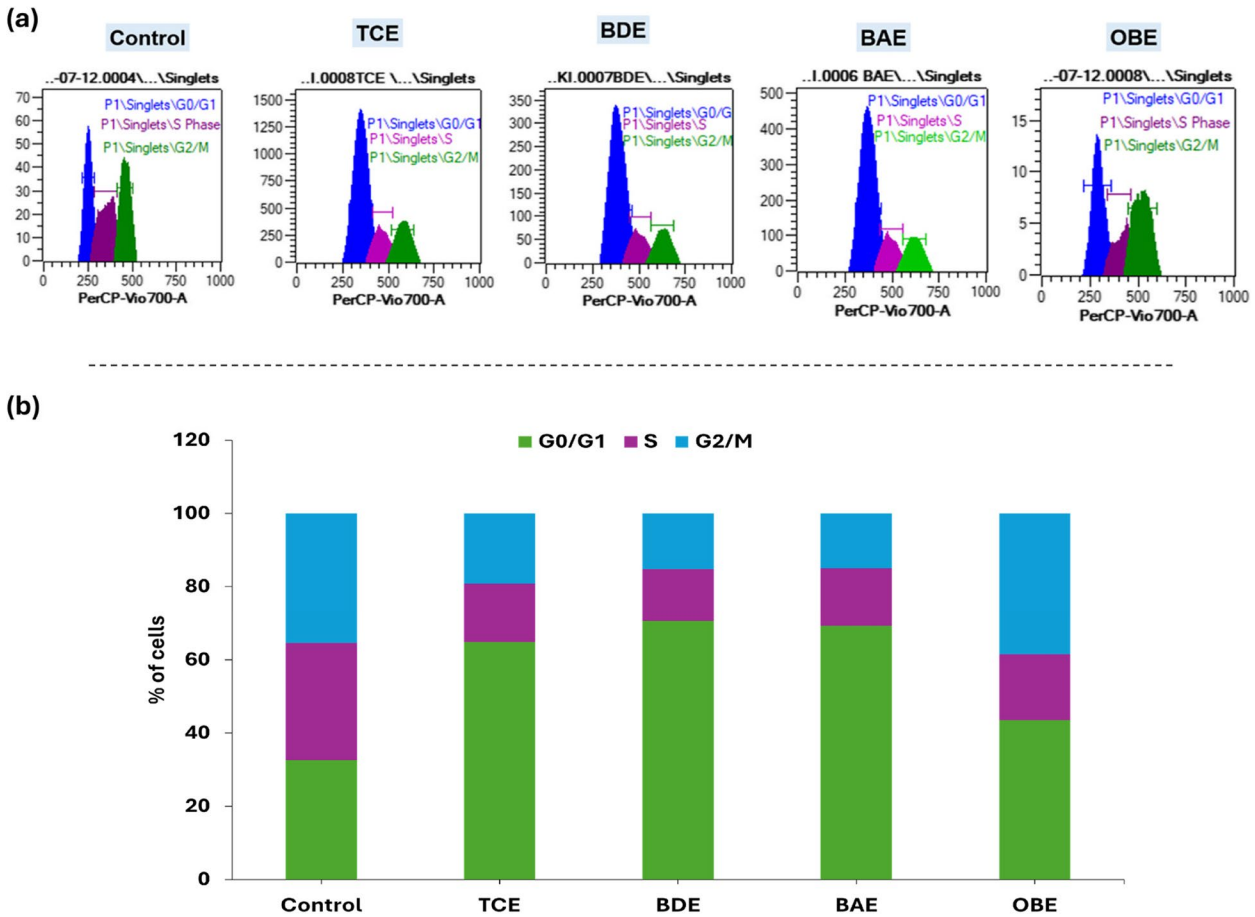

Fig. 3 Cell Cycle Analysis of CaSki Cells Treated with Plant Extracts (**a**) Flow cytometry histograms showing the distribution of CaSki cells in different phases of the cell cycle (G0/G1, S, and G2/M) following treatment with TCE, BDE, BAE, and OBE. The control group displays a normal cell cycle distribution. Treatment with the plant extracts induces significant cell cycle arrest, particularly at the G0/G1 phase. **b** Stacked bar graph representing the percentage of cells in each phase of the cell cycle across different treatment groups. The graph highlights the increased proportion of cells in the G0/G1 phase and the corresponding decrease in the S phase, indicative of cell cycle arrest. Data are expressed as mean ± SEM for three independent experiments [*Tinospora cordifolia* extract (TCE), *Boerhaavia diffusa* extract (BDE), *Berberis aristata* extract (BAE), and *Ocimum basilicum* extract (OBE)]

dose-dependent manner and promoting differentiation (Mishra and Kaur [22]). These observations reinforce the therapeutic potential of TCE, in targeting cell cycle dysregulation, a hallmark of cancer. However, further studies are necessary to elucidate the precise molecular mechanisms underlying these effects. Such investigations could involve evaluating the expression of key cell cycle regulators, such as p21, p27, and cyclins, to provide a more comprehensive understanding of their anticancer properties.

Evaluation of Genotoxic effects of Cisplatin and Plant extracts on HEK 293 cells

The genotoxic effects of CIS and the plant crude extracts were assessed on HEK 293 cells using the comet assay, a reliable method for detecting DNA damage at the individual cell level. Figure 4 visually captures the DNA damage in cells treated with each substance compared to the control, highlighting significant differences in the extent of DNA fragmentation.

In the assay, CIS, a conventional chemotherapeutic agent, induced pronounced DNA damage as evidenced by significantly longer DNA tail lengths in the treated cells (144.64 ± 21.10 pixels). This extensive damage aligns with the known genotoxic profile of CIS, which forms DNA adducts leading to significant strand breaks. In contrast, the plant extracts exhibited considerably shorter tail lengths, with TCE showing 43.91 ± 10.59 pixels, BDE at 44.82 ± 21.79 pixels, BAE at 17.36 ± 7.23 pixels, and OBE at 22.73 ± 6.69 pixels. These results suggest that these extracts are substantially less genotoxic than CIS. Notably, BAE demonstrated the least DNA damage among the extracts, indicating its potential as a milder alternative in therapeutic applications where DNA preservation is crucial. The mean values of tail length and tail DNA percentage are presented in graphical form as shown in Fig. 5, highlighting the statistical significance of the differences observed.

Compounds such as berberine and isoquinoline, extracted from *T. cordifolia*, demonstrate dose-dependent DNA damage in the HeLa cell line within 4 h of treatment (Jagetia and Rao [12]). While the precise mechanism of DNA damage induction remains unclear, it is speculated to involve cellular stress through the generation of ROS, free radicals, and lipid peroxidation (Rao [13]). *B. diffusa* known for its content of alkaloids, flavonoids, tannins, saponins, and terpenes, displays both antioxidant and anticancer activities, potentially shielding cells from ROS and mitigating the adverse effects of chemotherapeutic drugs. It also demonstrates anticancer activity in a dose-dependent manner on the SiHa cell line and inhibits the HeLa cell line during the S-phase of the cell cycle (Ameen et al. [1]). Berberine, a major alkaloid in *B. aristata*, exhibits inhibitory effects in a time and dose-dependent manner in the CaSki cell line, inducing ROS production and subsequent expression of GADD153, which disrupts cytochrome C release and activates caspase-3, leading to apoptosis (Lin et al. [19]). It also inhibits cell proliferation in various triple-negative breast cancer cell lines in a dose and time-dependent manner (Khalki et al. [15]). The findings of this study align with research on Pd(II) and Pt(II) trinuclear chelates, which highlighted the potential for synergistic interactions between chemotherapeutic agents and complementary treatments in overcoming resistance mechanisms (Vojtek et al. [41]).

The implications of these findings are significant, particularly in the context of developing less harmful

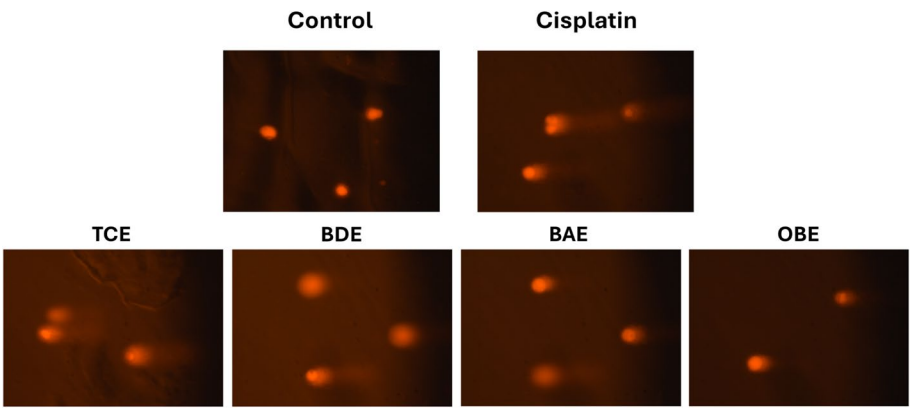

Fig. 4 Comet Assay Results of HEK 293 Cells Treated with Cisplatin and Plant Extracts TCE, BDE, BAE, and OBE. The images display the comet assay results for HEK 293 cells untreated (Control) and treated with cisplatin, TCE, BDE, BAE, and OBE. DNA fragmentation is visually less extensive in cells treated with plant extracts compared to those treated with cisplatin, highlighting their reduced genotoxicity [*Tinospora cordifolia* extract (TCE), *Boerhaavia diffusa* extract (BDE), *Berberis aristata* extract (BAE), and *Ocimum basilicum* extract (OBE)]

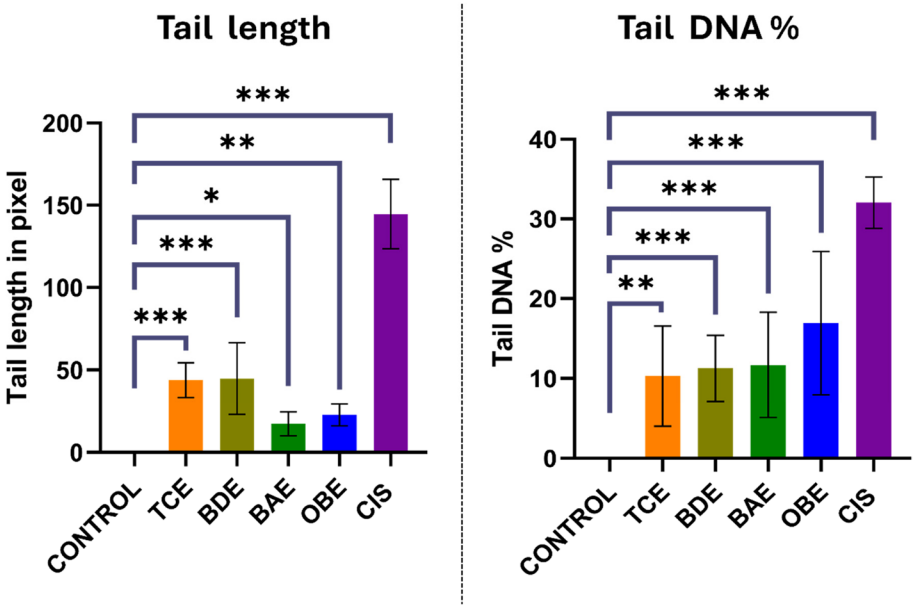

Fig. 5 Tail length and Tail DNA % of the comet performed of different plant extracts and chemotherapeutic drug cisplatin are represented in form of bar. Statistical significance is indicated by asterisks: * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$)

therapeutic alternatives to traditional chemotherapy agents like CIS. The markedly lower levels of DNA damage caused by the plant extracts suggest their potential utility in cancer therapy with reduced genotoxic side effects. BAE shows promise due to its minimal impact on DNA integrity, underscoring the importance of exploring plant-based compounds in the development of milder cancer treatment modalities.

Wound healing assay: analysis of cell migration in CaSki cells treated with plant extracts

The wound healing assay was conducted to check the effects of selected plant extracts on the migratory behaviour of CaSki cells, focusing on the potential influence on migratory capabilities. This assay offered insights into how alterations in cell viability and DNA integrity caused by plant extracts could affect the migratory properties of cervical cancer cells. Initial scratch areas were recorded at 0 h, serving as a baseline, and re-evaluated at 24 h post-treatment, as depicted in Fig. 6. The percentage of wound closure was calculated, with results presented in Fig. 7 and detailed in Table S3. Figure 7 demonstrates the comparative analysis of the percentage area of wound healing before and after giving the treatment with the plant extracts.

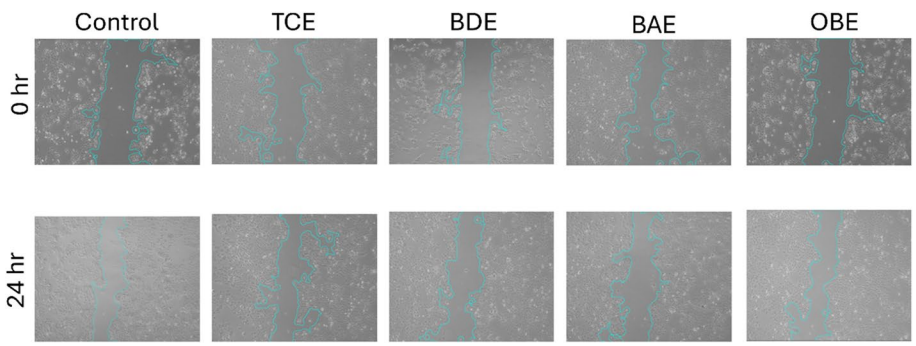

Fig. 6 Wound Healing Assay Images and Analysis of CaSki Cells Treated with Plant Extracts TCE, BDE, BAE, and OBE Illustrates the scratch wound healing assay results with images captured at 0 and 24 h post-treatment, showing the degree of wound closure in CaSki cells treated with different plant extracts compared to the control [*Tinospora cordifolia* extract (TCE), *Boerhaavia diffusa* extract (BDE), *Berberis aristata* extract (BAE), and *Ocimum basilicum* extract (OBE)]

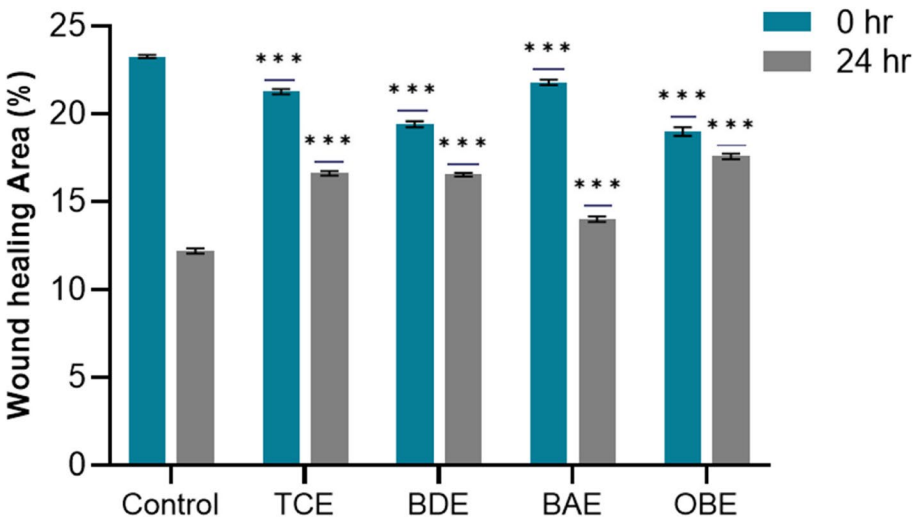

Fig. 7 The bar graph represents the percentage area of wound healing in CaSki cells after 0 h and 24 h of treatment with TCE, BDE, BAE, and OBE. The data compare the extent of wound closure between control and treated groups, illustrating the impact of each extract on cell migration and wound healing capabilities. Statistically significant differences between the control and treated groups are indicated by asterisks: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. [*Tinospora cordifolia* extract (TCE), *Boerhaavia diffusa* extract (BDE), *Berberis aristata* extract (BAE), and *Ocimum basilicum* extract (OBE)]

The control group exhibited the greatest reduction in scratch area, showing higher cell motility under normal conditions. In contrast, cells treated with the plant extracts displayed varying degrees of migration inhibition. TCE and OBE treatments resulted in moderate reductions in wound closure compared to the control, suggesting a slight suppression of cell migration. Notably, BDE and BAE treatments led to more pronounced inhibition, with BAE showing a significant decrease in cell migration ($p < 0.05$), highlighting its potential anti-metastatic effects at the tested concentrations.

Statistical analysis confirmed that the migration inhibition observed with BAE was significant ($p < 0.05$), whereas the effects of TCE and OBE were not statistically significant, indicating that these extracts did not markedly affect the migratory capacity of CaSki cells under the experimental conditions. Additionally, previous studies have shown that certain plant extracts can inhibit cancer cell migration in wound healing assays, supporting their potential role in anti-metastatic therapy (Wang et al. [42]).

These findings suggest that while certain extracts, particularly BAE, may possess properties that suppress cervical cancer cell migration, the overall impact at the tested concentrations was limited. This implies that higher concentrations or prolonged exposure might be necessary to fully realize the anti-migratory potential of these extracts. The differential effects on cell migration also indicate that these extracts could selectively modulate cellular behaviours, which is crucial for developing targeted therapies against metastatic cancer.

It is important to note that the IC_{50} doses used in this assay maintain consistency across all tests in this study. However, using cytotoxic doses can complicate the interpretation of migration effects, as it becomes challenging to determine whether reduced migration is due to inhibited migratory ability or simply a consequence of cell death. Future studies should address this limitation by employing non-cytotoxic doses to specifically assess cell migration without the influence of cytotoxicity, thereby providing a clearer understanding of the extracts' impact on metastatic potential.

Chemopreventive Effect of Plant extracts on HPV

Qualitative tests for HPV 16 confirmed that CaSki cells possess HPV 16 DNA, indicating their HPV-positive status. To assess the effects of plant treatments on HPV levels, quantitative PCR (qPCR) was performed. As shown in Table 2, the control (untreated) sample had the lowest CT value (20.11), indicating the highest initial concentration of HPV 16 DNA among the samples. All plant extract-treated samples showed higher CT values compared to the control, suggesting a reduction in the initial quantity of HPV 16 DNA to varying extents.

The data indicates that all tested plant extracts exert some inhibitory effect on the presence of HPV 16 DNA, with varying degrees of effectiveness. BDE was the most effective, showing the highest CT value of 24.17,

Table 2 Effect of plant extracts [*Tinospora cordifolia* extract (TCE), *Boerhaavia diffusa* extract (BDE), *Berberis aristata* extract (BAE), and *Ocimum basilicum* extract (OBE)] on HPV using q-PCR

Plant extract	Mean CT values of RT-PCR (HPV 16 copies)
Control	20.11 ± 0.1
TCE	22.80 ± 0.1
BDE	24.17 ± 0.3
BAE	23.74 ± 0.3
OBE	22.96 ± 0.8

which signifies the greatest reduction in HPV 16 DNA levels. This result aligns with previous findings on the antiviral properties of *B. diffusa*, where a glycoprotein isolated from the plant demonstrated broad-spectrum antiviral activity against various plant viruses (Awasthi et al. [2]). Although direct evidence of its effect against HPV is limited, the broad antiviral profile of *B. diffusa* suggests that its bioactive compounds could disrupt viral replication or promote viral clearance.

BAE also significantly reduced HPV 16 DNA levels, yielding a CT value slightly lower than BDE but higher than TCE. This reduction is consistent with the known antiviral properties of berberine, a major alkaloid in *B. aristata*. Berberine has been shown to inhibit HPV transcription and induce apoptosis in cervical cancer cells by modulating AP-1 activity (Mahata et al. [20]). Such mechanisms likely contribute to the observed reduction in HPV DNA in this study, reinforcing the potential of BAE as a complementary treatment for HPV-related cervical cancer.

These findings suggest that the plant extracts tested could potentially contribute to reducing HPV 16 levels, with BDE being the most promising candidate among those tested. The varying degrees of effectiveness among the extracts could be attributed to differences in their phytochemical compositions, which may influence their ability to inhibit viral replication or promote viral clearance. The observed reduction in HPV DNA levels indicates that these extracts might interfere with the viral life cycle or enhance the degradation of viral DNA, thereby contributing to the overall reduction in viral load. In conclusion, the observed reductions in HPV 16 DNA levels upon treatment with these plant extracts, particularly BDE and BAE, highlight their potential as complementary therapies in managing HPV-related cervical cancer. Further research is essential to elucidate their mechanisms of action, optimize their efficacy, and confirm their safety in clinical settings.

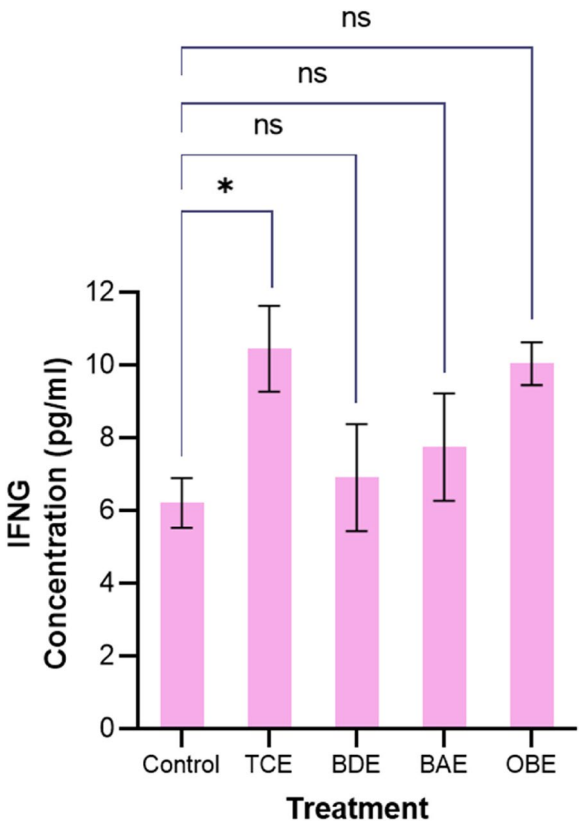

Fig. 8 The bar graph represents the concentration of IFNG (pg/ml) in CaSki cell culture supernatants after treatment with TCE, BDE, BAE, and OBE compared to the control. The fold change in IFNG levels is also shown, indicating the immunomodulatory effects of each extract. Statistically significant differences between the control and treated groups are indicated by asterisks: * $P < 0.05$, ns- non-significant. [*Tinospora cordifolia* extract (TCE), *Boerhaavia diffusa* extract (BDE), *Berberis aristata* extract (BAE), and *Ocimum basilicum* extract (OBE)]

Assessment of immunomodulatory properties

IFNG quantification assay

The immunomodulatory effects of plant extracts on CaSki cells were assessed by measuring the levels of IFNG in the cell culture supernatant using an ELISA kit. Following the treatment of CaSki cells with IC_{50} concentrations of the plant extracts for 24 h, the remaining supernatant was collected for IFNG quantification. The concentration of IFNG and the corresponding fold change relative to the control are shown in Table S4.

The results, as depicted in Fig. 8, indicate that the plant extracts have varying effects on the secretion of IFNG by CaSki cells. The control group, which was treated with the corresponding media without extracts, showed a baseline level of IFNG secretion. TCE and OBE treatments resulted in increase in IFNG levels, suggesting an enhancement of the immune response. BAE and BDE also increased IFNG levels,

but to a lesser extent than TCE and OBE. The differential effects of these plant extracts on IFNG production could be attributed to the various bioactive compounds they contain, which may interact differently with the immune system.

The observed increase in IFNG concentration following TCE treatment is consistent with existing literature. Studies have shown that *T. cordifolia* can modulate the immune system by increasing IFNG production, thereby enhancing the body's defence mechanisms (Kumar et al. [17]). Similarly, the immunomodulatory effects of *B. diffusa* have been documented, with reports indicating its ability to influence cytokine production and stimulate immune responses (Vidhya et al. [40]). This aligns with findings by previous study in which they have reported that polyphenolic compounds like curcumin significantly induced IFNG production by CD4 + T cells in colon cancer patients. Such modulation of IFNG levels underscores the potential of bioactive compounds in these extracts to enhance antitumor immunity (Bahrami et al. [3]).

The role of helper T cells (CD4 + CD3+), known to secrete IFNG and orchestrate immune responses, is pivotal in this context. Our findings are consistent with the study by Deng and colleagues, which highlights the critical role of CD4 + T cells in mediating tumor immunity and suggests that targeting immune checkpoints and regulatory pathways could enhance T cell-mediated cytotoxicity against tumors (Deng et al. [8]). Moreover, another study emphasized that natural products derived from herbal medicines can modulate immune cell activity by increasing CD4 + T cell populations, consistent with our observation of enhanced helper T cell activity following treatment with these extracts (Zhong et al. [46]). In line with our findings, the review article underscores the potential of natural products in cancer immunotherapy by enhancing cytotoxic T cell activity, modulating cytokine production, and inhibiting immune checkpoints like PD-1/PD-L1 (Nuzzo et al. [25]). This complements our results, showing significant modulation of CD3 + and CD4 + markers, suggesting the activation of T cells and improved antitumor immune responses.

While this study focused on IFNG due to its central role in the immune response to cancer cells and its relevance in HPV-positive cervical cancer, additional cytokines, such as IL-7, could provide further insights into the mechanisms of immune modulation. IFNG and IL-7 have interconnected roles, particularly in enhancing T-cell survival and function, which are essential in antitumor immunity. Future studies should include IL-7 measurements to explore these extracts' effects on T-cell survival and broader immune functions, offering a more comprehensive understanding of their immunomodulatory potential.

Flow cytometry: analysis of CD markers

The immunomodulatory effects of plant extracts were evaluated by examining the distribution of different T cell subsets derived from PBMCs using flow cytometry. After treatment with the plant extracts, the percentages of various CD markers in various immune cell subsets were assessed to determine the influence of these extracts on the immune cell populations. The results are summarized in Table S5 and visually represented in Figs. 9 and 10.

CD45 + cells represent a broad marker for leukocytes, including T cells. Variations in the percentage of CD45 + cells after treatment with plant extracts would indicate changes in the overall T cell or leukocyte population. TCE treatment modulated the highest percentage of CD45 + cells, suggesting a significant effect on the leukocyte population. This aligns with previous studies demonstrating *T. cordifolia*'s ability to enhance leukocyte counts and modulate immune responses (Shivananjappa [36]).

Helper T cells, identified as CD4 + CD3 + cells, are crucial for orchestrating the immune response. An increase in this population, particularly with OBE treatment, suggests that the plant extract can enhance the adaptive immune system by promoting T cell proliferation or reducing T cell apoptosis. This is particularly relevant in the context of cervical cancer, where immune evasion plays a significant role in cancer progression and HPV persistence. CD4 + cells include a broader range of T cells, not exclusively those co-expressing CD3. TCE treatment notably increased the percentage of CD4 + cells, suggesting that TCE can enhance the population of helper T cells, thus potentially improving immune response.

CD19 + and CD19 + CD56 + cells identify B cell populations and a subset of B cells co-expressing NK markers, respectively. Changes in these populations would indicate effects on humoral immunity and interactions between the innate and adaptive immune systems. TCE treatment increased the percentage of CD19 + CD45 + cells, suggesting a potential role in enhancing humoral immunity. Representing NK cells, CD56 + cells would indicate effects on innate immunity. While not T cells, NK cell modulation is crucial for initial cancer cell recognition and destruction. The Table S5 shows negligible changes in this category, suggesting minimal impact on NK cells by the plant extracts.

In summary, the plant extracts, particularly TCE and OBE, modulated immune cell populations by enhancing helper T cell proliferation and potentially augmenting the immune system's capacity to target cancer cells. This dual action—directly targeting cancer cells and indirectly supporting antitumor immunity—highlights their potential as complementary agents in cancer therapy. Further

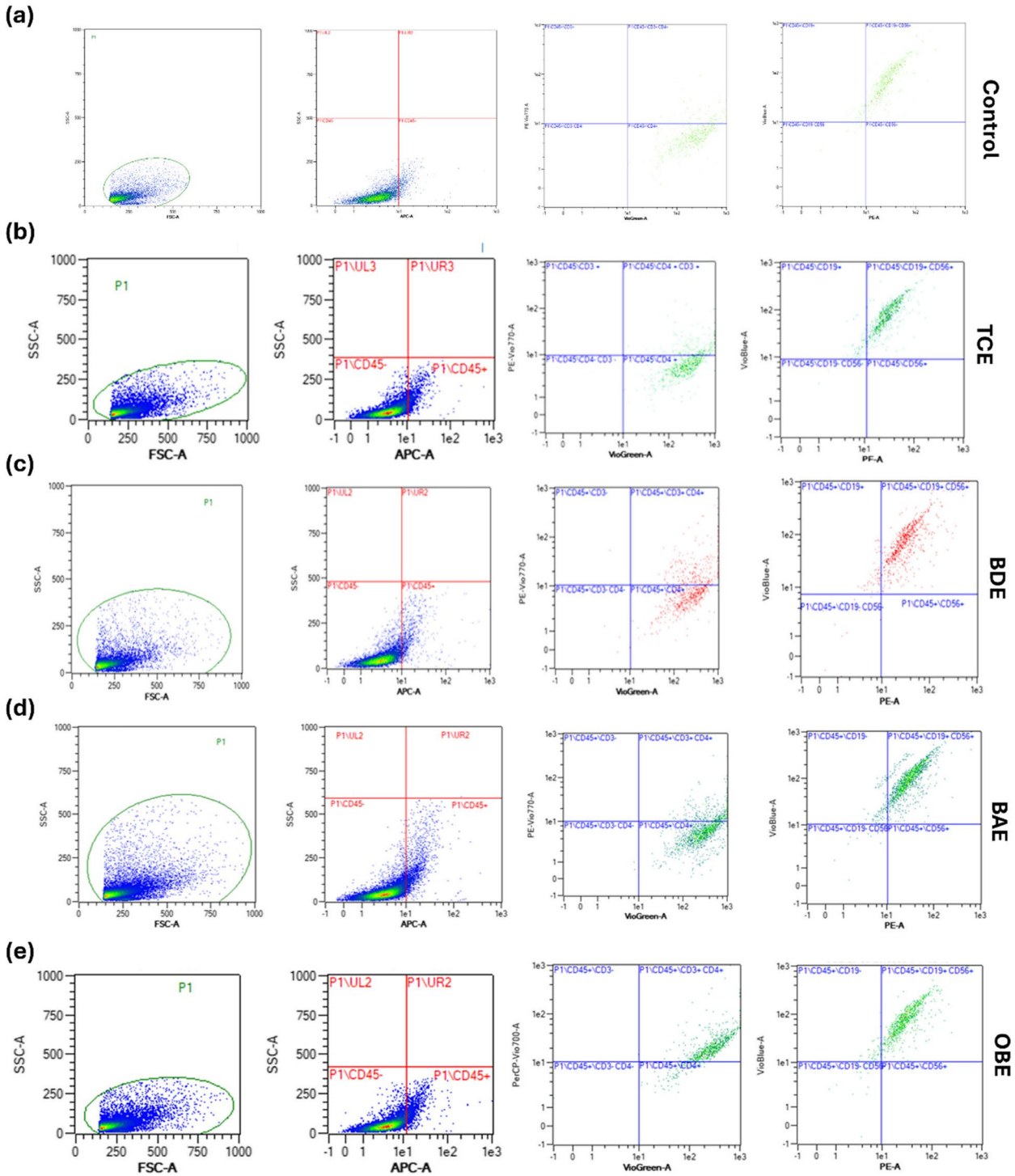

Fig. 9 Various immune cell subset analysis of (**a**) Control (**b**) TCE, (**c**) BDE, (**d**) BAE, and (**e**) OBE by flow cytometry. The analysis revealed that the plant extracts TCE, BDE, BAE, and OBE influenced the proliferation and activation of various immune cell subsets. TCE and OBE significantly promoted the proliferation of different immune cell subsets, suggesting a broader immunomodulatory effect [*Tinospora cordifolia* extract (TCE), *Boerhaavia diffusa* extract (BDE), *Berberis aristata* extract (BAE), and *Ocimum basilicum* extract (OBE). %T indicates the total percentage cells of the gated population]

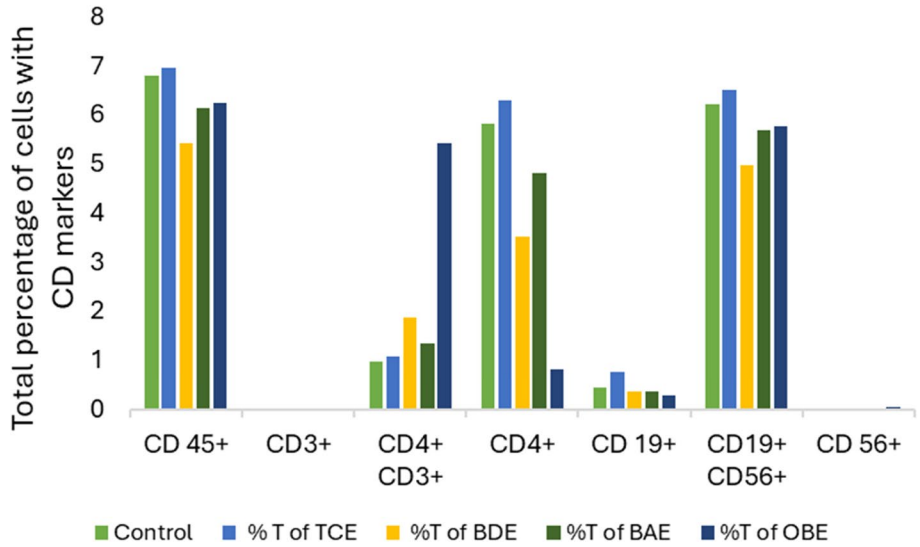

Fig. 10 Total Percentage of cells with Selected CD Markers after Treatment with Plant Extracts The bar graph represents the percentage of different immune cell subsets expressing selected CD markers (CD45+, CD3- CD4-, CD3+, CD4 + CD3+, CD4+, CD19- CD56-, CD19+, CD19 + CD56+, CD56+) after treatment with TCE, BDE, BAE, and OBE. The data illustrate the impact of the plant extracts on the proliferation and activation of various immune cell populations [*Tinospora cordifolia* extract (TCE), *Boerhaavia diffusa* extract (BDE), *Berberis aristata* extract (BAE), and *Ocimum basilicum* extract (OBE). %T indicates the total percentage cells of the gated population]

research is warranted to elucidate the specific mechanisms underlying these effects and to assess their clinical relevance.

Conclusion

This study underscores the potential of medicinal plant extracts—*Tinospora cordifolia* (TCE), *Boerhaavia diffusa* (BDE), *Berberis aristata* (BAE), and *Ocimum basilicum* (OBE) as viable alternatives to traditional chemotherapy in treating HPV-positive cervical cancer. These extracts showed cytotoxic effects against cancer cells, with limited impact on normal cells, suggesting a favorable safety profile that may reduce the side effects commonly associated with conventional cancer treatments. Among the extracts, BAE exhibited notable anticancer activity and minimal genotoxicity, indicating its suitability for therapeutic applications. The immunomodulatory properties observed, particularly the increase in IFNG production following TCE and OBE treatment, suggest that these extracts may also support immune responses, potentially enhancing the natural ability of body to combat cancer.

To build on these findings, future research should focus on fractionating these extracts and identifying the specific bioactive compounds responsible for their effects, using techniques such as HPLC and LC-MS. Additionally, in vivo studies are recommended to further validate the therapeutic potential and safety profile of these extracts in a biological system. In clinical contexts, integrating these plant extracts into existing cancer treatment protocols may provide more accessible and cost-effective therapeutic options, particularly for resource-limited settings. However, further preclinical and clinical evaluations are necessary to confirm their efficacy and safety in humans.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12906-025-04817-x.

Supplementary Material 1

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Authors' contributions

The experiments were conceptualized by R.R. and H.V.; data curation was managed by H.V. The genotoxicity analysis assay was performed by R.P., who also assisted in all other experiments. K.S. has performed the cell cycle and immunomodulation assays. The data analysis and manuscript preparation were collaboratively handled by H.V., J.P., R.P., and K.S. Supervision of the research and revisions of the manuscript were overseen by R.R. and M.S. All authors critically discussed the manuscript, contributing important intellectual content, and approved the final version for publication.

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Data availability
All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
This study was reviewed and approved by the Institutional Ethics Committee of Gujarat University, Ahmedabad, Gujarat, India (Approval Number: GU/IEC/11/2019). The study involved the use of discarded umbilical cord blood samples, and the need for informed consent was waived by the ethics committee as per the guidelines.

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

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