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Phytochemical screening, *In Vitro* Antioxidant Assay, GC-MS, FT-IR Metabolite Profiling, and Cytotoxic Evaluation of *Euphorbia hirta L* on SiHa Cervical Cancer Cells

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KEYWORDS

Euphorbia hirta; Phytochemical screening; Antioxidant activity; DPPH assay; Reducing power assay; GC–MS analysis; FT-IR spectroscopy; HPTLC profiling; Cytotoxicity; SiHa cervical cancer cell line; MTT assay; Bioactive compounds.

ABSTRACT:

Ayurveda, the classical system of traditional Indian medicine, encompasses eight specialized branches that focus on the therapeutic properties of drugs, preventive healthcare, and holistic approaches to healing. Euphorbia hirta is a widely used medicinal plant known for its diverse ethnopharmacological applications. The present study aimed to investigate the phytochemical composition, antioxidant potential, cytotoxic activity, and bioactive constituents of the methanolic extract of Euphorbia hirta using GC–MS, HPTLC, and FT-IR analyses. Preliminary phytochemical screening confirmed the presence of tannins, alkaloids, saponins, flavonoids, terpenes, anthraquinones, and steroids. The extract exhibited significant antioxidant activity in both DPPH radical scavenging and reducing power assays, showing concentration-dependent effects with IC_{50} values of 16.5 µg/mL and 52.5 µg/mL, respectively. GC-MS analysis revealed multiple phytoconstituents, with 3-penten-2-one and 4-methyl identified as the predominant compounds. HPTLC profiling revealed distinct bands, confirming the phytochemical diversity, while FT-IR analysis identified characteristic functional groups, including O–H, C–H, and C=O, indicating the presence of phenolic and oxygenated compounds. The extract also exhibited moderate cytotoxic activity against the SiHa cervical cancer cell line, with an IC_{50} value of approximately 108 µg/mL. Collectively, these findings suggest that Euphorbia hirta possesses significant antioxidant and cytotoxic potential, supporting its traditional medicinal use and highlighting its promise as a source of bioactive compounds for therapeutic development.

Introduction

The therapeutic use of natural products, particularly medicinal plants and their derivatives, can be traced back to the earliest stages of human civilization. One of the oldest documented records of plant-based medicine appears in the *Rigveda* (4500–1600 B.C.), highlighting the long-standing reliance on botanical resources for healthcare. Ayurveda, the traditional Indian system of medicine, comprises eight distinct branches that emphasize drug properties, disease prevention, and holistic healing (Rastogi and Mehrotra, 2002). Owing to their rich phytochemical diversity, medicinal plants represent a valuable reservoir of bioactive compounds with diverse pharmacological activities. Approximately 80% of the global population continues to depend on plant-based medicines for primary healthcare (Ghosh *et al*., 2019 a). According to the World Health Organization (WHO), nearly 75% of the global population depends on herbal and traditional medicines for disease treatment. Medicinal plants contain diverse bioactive compounds such as phenols, tannins, alkaloids, flavonoids, essential oils, and peptides, which exhibit broad-spectrum

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antimicrobial activity against bacteria, fungi, and viruses (Ghosh *et al*., 2019 b).

In recent years, increasing scientific attention has been directed toward the systematic evaluation of medicinal plants to validate their traditional claims and identify novel bioactive constituents with therapeutic potential. Among such plants, *Euphorbia hirta* has attracted considerable interest due to its wide spectrum of ethnomedicinal applications and reported pharmacological properties. *Euphorbia hirta* L., a medicinal herb belonging to the family Euphorbiaceae, is commonly referred to as the "asthma plant" and is known by various vernacular names across different regions. The plant typically grows in open wastelands, grasslands, roadsides, and along pathways. Although native to Central America, *E. hirta* is widely distributed and cultivated throughout tropical regions, particularly in West, Central, and East Africa (Iskandar *et al*., 2022; Silalahi, 2021). Various parts of *Euphorbia hirta* L., particularly the leaves and roots, have been extensively utilized in traditional medicine as decoctions and infusions for the treatment of diverse ailments, including skin disorders, toothache, wounds, headache, rheumatism, diarrhea, dysentery, colic, gonorrhea, migraines, and pregnancy-related pain, as well as to promote lactation (Rajesh *et al*., 2010). The plant has been reported to exhibit a wide spectrum of biological activities such as analgesic, anti-arthritic, antipyretic, anti-inflammatory, antimicrobial, antioxidant, antitumor, anthelmintic, anticancer, diuretic, sedative, and anxiolytic effects (Tran *et al*., 2020; Youssouf *et al*., 2007; Asha *et al*., 2016; Karki *et al*., 2020). Owing to these pharmacological properties, *E. hirta* has gained considerable attention and is widely employed in the management of asthma, bronchitis, cancer, diabetes, kidney stones, dengue, intestinal disorders, and ocular infections (Widharna *et al*., 2010; Khurshid *et al*., 2013; Guzman *et al*., 2016). *Euphorbia hirta* is rich in diverse bioactive secondary metabolites, including flavonoids (quercetin, myricetin), sterols (β-sitosterol, cycloartenol), tannins (euphorbins, gallic acid derivatives), and triterpenoids (α- and β-amyrin, taraxerone), along with phenolic acids and fatty acids. These compounds are widely recognized for their antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and cytotoxic activities, collectively supporting the plant's broad therapeutic potential (Kumar *et al*., 2010).

Cervical cancer remains a significant global public health concern, ranking as the fifth most common cancer in terms of incidence and the fourth leading cause of cancer-related mortality among women worldwide. The burden is particularly alarming in the South Asian region, especially in India, where cervical cancer is the second most prevalent cancer among women. According to GLOBOCAN 2022 estimates, India reported approximately 127,526 new cases and 79,906 deaths attributed to cervical cancer in 2022 (IARC, 2022; Singh *et al*., 2025). Venkatajothi et al. (2010) reported HPV-16 as the most prevalent genotype among HIV-positive women in Tanzania, underscoring its major role in cervical cancer development. Given the strong association between HPV-16 and cervical malignancy, exploring novel therapeutic agents against cervical cancer is crucial. In this context, the present study evaluates the cytotoxic potential of Euphorbia hirta methanolic extract against the SiHa cervical cancer cell line, a model associated with HPV-related carcinogenesis.

Euphorbia hirta L. may also have anticancer activities. *E. hirta* has previously been demonstrated to inhibit the growth of B16F10 melanoma cells, the HepG2 liver cancer cell line, the HT-29 human colon cancer cell line, and MCF-7 breast cancer cells. However, *E. hirta* extracts have never been tested on SiHa cervical cancer cells.

To address this research gap, the present study aimed to evaluate the cytotoxic efficacy of the methanolic extract of *Euphorbia hirta* against the SiHa cervical cancer cell line using the MTT assay. Furthermore, comprehensive phytochemical profiling was performed through preliminary screening, GC–MS, HPTLC, and FT-IR analyses to identify potential bioactive constituents responsible for the observed anticancer activity. By integrating phytochemical characterization with biological evaluation, this study seeks to provide mechanistic insights into the therapeutic potential of *E. hirta* and to support its development as a promising plant-derived candidate for cervical cancer management.

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Materials and methods

Sample Collection and authentication

The whole plant of *Euphorbia hirta* was collected from agricultural land in Salem, Tamil Nadu, India. The collected specimen was authenticated by Dr. V. Balasubramaniam, Senior Agricultural Officer, Tamil Nadu Agricultural University (TNAU), in Coimbatore. The authenticated plant material was used for subsequent experimental analysis.

Preparation of extract

The collected plants were subjected to extraction. Briefly, the plant materials were washed and placed in a drying oven at 42°C overnight. The dried plant materials were then ground to small particles using a domestic blender. Then, the solvent (methanol) was added in the proportion of 10 g in 100 mL solvent to the flasks. The mixture was left on a shaker set at 100 rpm and ran for 16 h at 30°C to macerate. The mixture was decanted through Whatman filter paper, and the filtrate was collected and concentrated by a vacuum rotary evaporator (Heidolph Rotavac, Germany). The stock solution of plant extracts (50 mg/mL) was filtered through a 0.22-µm polyethersulfone filter membrane (Millipore, USA) and serially diluted into several working solution concentrations in culture medium. Both stock and working solutions of plant crude extracts and cisplatin were stored at −20°C until further use (Ismail *et al*., 2019).

Phytochemical analysis of the methanolic extract of *E. hirta*

Preliminary phytochemical analysis of the methanolic and hexane extracts of *Euphorbia hirta* was carried out using standard qualitative methods as described by Edeoga *et al*. (2005), Trease and Evans (1996), Harborne (1984), Daniel (2006), and Prashith Kekuda *et al*. (2012).

Test for Tannins:

A small quantity of the extract was treated with 10% alcoholic ferric chloride solution. The formation of a dark blue or greenish-black coloration indicated the presence of tannins.

Test for Alkaloids (Dragendorff's Test):

To 1 mL of the extract solution, a few drops of Dragendorff's reagent were added. The appearance of an orange precipitate confirmed the presence of alkaloids.

Test for Saponins (Foam Test):

The crude extract was mixed with 5 mL of distilled water and shaken vigorously. The formation of a stable and persistent froth indicated the presence of saponins.

Test for Flavonoids:

The extract was treated with a few drops of sodium hydroxide solution. The development of an intense yellow coloration that turned colorless upon addition of dilute acid confirmed the presence of flavonoids.

Test for Terpenoids (Salkowski Test):

Approximately 0.2 g of the extract was mixed with 2 mL of chloroform, followed by the careful addition of 3 mL of concentrated sulfuric acid to form a separate layer. A reddish-brown coloration at the interface indicated the presence of terpenoids.

Test for Anthraquinones:

About 0.5 g of the extract was boiled with 10% hydrochloric acid in a water bath for several minutes, filtered, and allowed to cool. The filtrate was extracted with chloroform, and a few drops of 10% ammonia solution were added. The appearance of a rose-pink coloration indicated the presence of anthraquinones.

Test for Steroids (Liebermann–Burchard Test):

The crude extract was dissolved in 0.5 mL of dichloromethane, followed by the addition of 0.5 mL of acetic anhydride and a few drops of concentrated sulfuric acid. The formation of a blue-green coloration confirmed the presence of steroids.

Antioxidant activity of the *E. hirta* methanolic extract

DPPH Radical Scavenging Assay

The antioxidant activity of the extract was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay, following the method described by Tran *et al*., (2020). DPPH is a stable free radical exhibiting a deep violet color with a

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characteristic absorption maximum at 515–517 nm. Upon reaction with an antioxidant capable of donating a hydrogen atom, DPPH is reduced to its non-radical form (DPPH), resulting in a color change from purple to yellow. The extent of radical scavenging activity was determined by measuring the decrease in absorbance at 515 nm.

Briefly, 0.5 mL of the plant extract at different concentrations (10, 50, 100, 250, 500, 750, and 1000 µg/mL) or ascorbic acid (0.01–0.5 mM) as the standard was mixed with 0.5 mL of 0.6 mM DPPH solution prepared in methanol. The final volume was adjusted to 4 mL with methanol. The reaction mixture was vortexed and incubated in the dark at room temperature for 30 min. Absorbance was recorded at 515 nm using a UV–Visible spectrophotometer, with methanol serving as the blank. A control was prepared by mixing 0.5 mL of DPPH solution with 3.5 mL of methanol. All experiments were performed in triplicate.

The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

Reducing power determination

The reducing power of the extract was determined based on its ability to reduce ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions, which serves as an indicator of electron-donating capacity and reflects the potential antioxidant activity of phenolic compounds. The assay was performed according to the method described by Dharmishtha *et al*. [11], with minor modifications. Briefly, 1 mL of the leaf extract at different concentrations (31–1000 µg/mL) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium hexacyanoferrate [$K_3Fe(CN)_6$]. The reaction mixture was incubated at 50 °C for 20 min. Subsequently, 2.5 mL of 10% trichloroacetic acid was added to terminate the reaction, and the mixture was centrifuged at 3000 rpm for 10 min (Hettich Zentrifugen, Universal 320R). The supernatant (2.5 mL) was then mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride ($FeCl_3$). The absorbance was measured at 700 nm using a UV–Visible spectrophotometer. A sample-free mixture served as the blank, while ascorbic acid was used as the positive control. All experiments were conducted in triplicate. An increase in absorbance at 700 nm indicated higher reducing power of the extract.

GC-MS analysis of the *E. hirta* methanolic extract

Gas chromatography–mass spectrometry (GC–MS) analysis of the methanolic extract of *Euphorbia hirta* was performed using a GCMS-QP 2010 Ultra system equipped with an Rtx-5MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness). The instrument was operated in electron ionization (EI) mode at 70 eV, with helium serving as the carrier gas. A volume of 1 µL of the methanolic extract of the whole plant was injected in splitless mode.

The column head pressure was maintained at 68.3 kPa. The oven temperature was programmed at 250 °C, 280 °C, and 300 °C, with respective hold times of 1.0, 2.0, and 10.0 minutes. The GC–MS interface temperature was set at 280 °C. Mass spectra were recorded in full scan mode within the mass range of 30–600 m/z. The total run time was 3.00–25.00 minutes. Identification of the compounds was achieved by comparing the obtained mass spectra with those available in the NIST (USA) and Wiley spectral libraries (Karki *et al*., 2020).

HPTLC Finger Prints of the *E. hirta* methanolic extract

High-performance Thin Layer Chromatography (HPTLC) was carried out for the extract of *E. hirta*. 20µl concentrations were applied on pre-coated silica gel GF254 HPTLC plates with Toluene: Ethyl acetate: Hexane: Formic acid (3:4:3:0.2) solvent system and scanned at 254 nm.

The data gathered were statistically analyzed using SPSS (Statistical Package for the Social Sciences) software version 24.0 (IBM Corp., Armonk. NY, USA) for assessment of descriptive measures, Student t-test, ANOVA (analysis of variance), Fisher's exact test, Mann-Whitney U test, and Chi-square test. The results were expressed as mean and standard deviation and frequency and percentages. The p-value of < 0.05 was considered.

FT-IR analysis of the *E. hirta* methanolic extract

Fourier Transform Infrared (FT-IR) spectroscopy was performed to identify the functional

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groups present in the chloroform, n-butanol, and ethyl acetate fractions of the methanolic extract of *Euphorbia hirta*. The powdered samples were analyzed using an FT-IR spectrophotometer (Shimadzu, model IR Prestige-21). Spectral data were recorded over a scanning range of 4000–400 cm^{-1} (Karki *et al*., 2020).

Cytotoxic effect against SiHa Cervical cancer cell line (MTT Assay)

Principle

MTT (3-4, 5 dimethylthiazol-2yl-2, 5-diphenyl tetrazolium bromide) assay, is based on the ability of a mitochondrial dehydrogenase enzyme of viable cells to cleave the tertrazolium rings of the pale yellow MTT and form a dark blue colored formazan crystal which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. Solubilization of cells by the addition of detergents (DMSO) results in the liberation of crystals which are solubilized. The number of surviving cells is directly proportional to the level of formazan product created. The color can be quantified using a multi-well plate reader.

Materials required

Fetal Bovine Serum (FBS) and antibiotic solution were from Gibco (USA), DMSO (Dimethyl sulfoxide) and MTT (3-4, 5 dimethylthiazol-2yl-2, 5-diphenyl tetrazolium bromide) (5 mg/ml) were from Sigma, (USA), DMEM medium, 1X PBS, (India). 96 well tissue culture plate and wash beaker were from Tarson (India).

Procedure

Cell culture

SiHa Cervical cancer cell lines were purchased from NCCS, Pune and were cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 µg/ml penicillin and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37ºC**.**

MTT assay

The test sample was tested for *in vitro* cytotoxicity, using SiHa cells by MTT assay. Briefly, the cultured SiHa cells were harvested by trypsinization and pooled in a 15 ml tube. Then, the cells were plated at a density of 1×10^5 cells/ml cells/well (200 µL) into the 96-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 hour at 37°C. The wells were washed with sterile PBS and treated with various concentrations of the test sample in a serum-free DMEM medium. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO2 incubator for 24 hours.

After incubation, MTT (10 µL of 5 mg/ml) was added to each well and the cells were incubated for another 2-4 hours until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT (220 µL) was aspirated off the wells and washed with 1X PBS (200 µl). Furthermore, to dissolve formazan crystals, DMSO (100 µL) was added, and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a microplate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC50 value were calculated using Graph Pad Prism 6.0 software (USA).

Formula Cell viability % = Test OD/Control OD X 100

Results

Medicinal plants have long served as an essential component of traditional healthcare systems, and recent years have witnessed a marked increase in scientific investigations aimed at validating their therapeutic efficacy. In this context, the present study evaluated the phytochemical composition and biological activities of *Euphorbia hirta* to substantiate its medicinal relevance.

Phytochemical screening

Qualitative phytochemical analysis of the methanolic extract of *Euphorbia hirta* revealed the presence of multiple bioactive secondary metabolites. The extract tested positive for tannins, alkaloids, saponins, flavonoids, terpenes, anthraquinones, and steroids (Table 1). The consistent positive reactions observed across all tested phytochemical groups indicate that the methanolic extract contains a diverse range of secondary metabolites. The presence of these phytoconstituents suggests that the extract possesses significant pharmacological potential and antioxidant activities.

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Table 1: Preliminary phytochemical analysis of the Methanolic extract of *E. hirta*

S. No	Test	Methanolic extract of *E. hirta*
1.	Tannins	Positive
2.	Alkaloids	Positive
3.	Saponins	Positive
4.	Flavonoids	Positive
5.	Terpenes	Positive
6.	Anthroquinones	Positive
7.	Steriods	Positive

Antioxidant activities of the methanolic extract of *E. hirta*

DPPH Radical Scavenging Activity

The antioxidant potential of the methanolic extract of *Euphorbia hirta* was evaluated using the DPPH radical scavenging assay. The extract demonstrated a concentration-dependent increase in free radical scavenging activity. At 20 µg/mL, the extract exhibited 6.1% inhibition, which progressively increased to 38.0% at 100 µg/mL. Similarly, the standard (ascorbic acid) showed higher scavenging activity across all tested concentrations, with percentage inhibition ranging from 8.7% at 20 µg/mL to 43.7% at 100 µg/mL (Table 2). The calculated IC_{50} value of the extract was 16.5 µg/mL, whereas the standard showed an IC_{50} value of 21.3 µg/mL. The lower IC_{50} value of the extract indicates a comparatively strong radical scavenging potential. Although the standard exhibited comparatively greater antioxidant activity, the extract displayed appreciable radical scavenging potential, indicating the presence of bioactive compounds capable of donating hydrogen atoms to neutralize DPPH radicals. These findings suggest that the methanolic extract of *Euphorbia hirta* possesses moderate antioxidant activity in a dose-dependent manner.

Table 2: ***In vitro* DPPH activity of *E. hirta* methanolic extract**

S.No	Concentration µg/ml	% Inhibition of Extract	% Inhibition of Standard
1.	20	6.1	8.7
2.	40	10.7	14.2
3.	60	23.1	26.4
4.	80	26.7	31.0
5.	100	38.0	43.7
IC_{50} Value of *E. hirta*		16.5	21.3

Reducing power assay of *E. hirta* methanolic extract

The methanolic extract of *Euphorbia hirta* demonstrated a concentration-dependent increase in reducing power activity. The percentage inhibition increased from 22.1% at 20 µg/mL to 84.7% at 100 µg/mL. Similarly, the standard (ascorbic acid) exhibited higher reducing activity, ranging from 26.7% at 20 µg/mL to 88.6% at 100 µg/mL (Figure 1).

The IC_{50} value for the extract was determined to be 52.5 µg/mL, whereas the standard showed an IC_{50} value of 57.5 µg/mL. The relatively lower IC_{50} value of the extract indicates appreciable electron-donating capacity and significant reducing potential. These findings suggest that the antioxidant activity of *Euphorbia hirta* may be attributed to the presence of phenolic and flavonoid compounds capable of reducing ferric ions.

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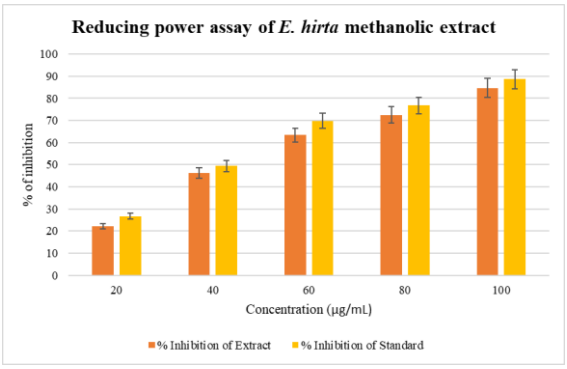

Figure 1: Reducing power assay of *E. hirta* methanolic extract

GC-MS analysis

GC–MS analysis of the methanolic extract of *Euphorbia hirta* revealed the presence of multiple phytochemical constituents with varying retention times and peak areas. A total of several compounds were tentatively identified through comparison with the NIST spectral library. The chromatogram showed a predominant peak at RT 16.34 min with the highest peak area (92.44%), corresponding to 3-penten-2-one, 4-methyl-, indicating it as the major constituent of the extract. Another significant compound was detected at RT 12.89 min with a peak area of 2.51%, identified as 3-penten-2-one, 4-methyl- and related derivatives.

A moderate peak at RT 31.80 min (2.16%) corresponded to bicyclo[3.1.1] heptane derivatives, while additional minor compounds were detected at RT 32.61, 33.11, 35.83, and 37.18 min (Figure 2). Other identified constituents included phenolic derivatives (phenol, 4-(1-methylethylidene)-), zirconium and iron-associated compounds, distannoxane derivatives, benzaldehyde derivatives, and phosphonic acid compounds. The presence of phenolic and ketonic structures suggests potential antioxidant properties, whereas bicyclic and aromatic derivatives may contribute to the cytotoxic activity observed in the extract.

The variation in peak area percentages reflects the relative abundance of compounds within the extract, with the compound at RT 16.34 min representing the major bioactive constituent. These findings support the phytochemical complexity of *Euphorbia hirta* and provide a chemical basis for its observed biological activities.

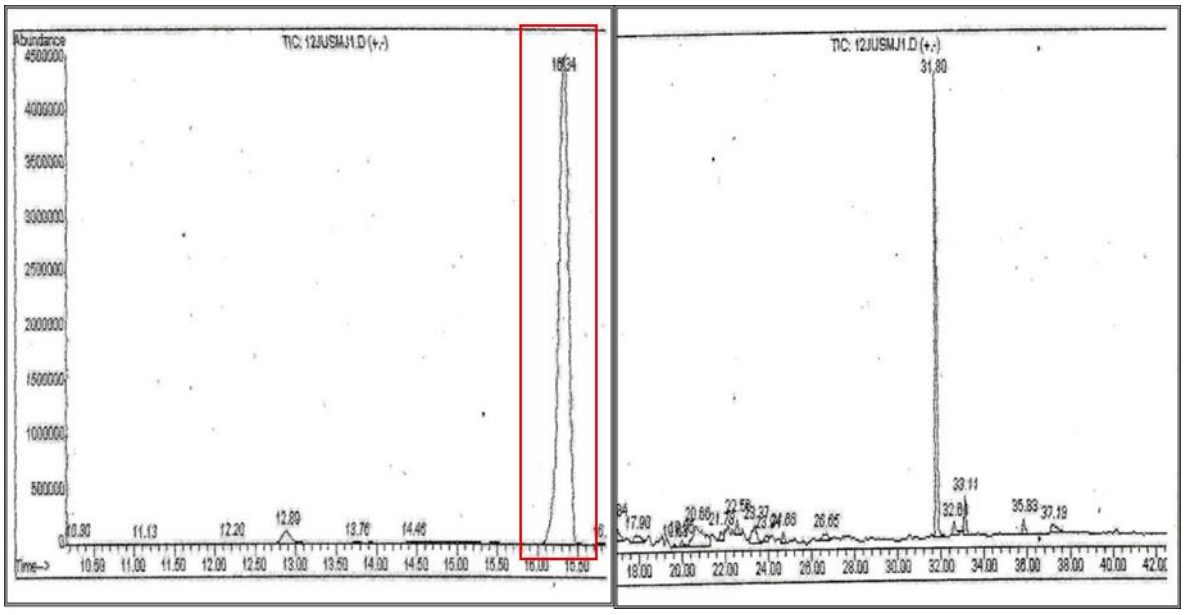

Figure 2: GC-MS analysis of the *E. hirta* methanolic extract

HPTLC Finger Prints of the extract of *E. hirta*

High Performance Thin Layer Chromatography (HPTLC) was carried out for the extract of *E. hirta*. 20µl concentrations were applied on pre-coated silica gel GF254 HPTLC plates with Toluene: Ethyl acetate: Hexane: Formic acid (3:4:3:0.2) solvent system and scanned at 254 nm. The methanol extract gave 8 peaks. The Rf values and peak areas were also measured, and

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the chromatogram is shown in Figure 3. The spot number (S. No) 7 with Rf value 0.84 was found to be higher in the methanolic extract of *E. hirta*.

TLC profiles of the extract of *E. hirta* on Silica gel "G" plate using Toluene: Ethyl acetate: Hexane: Formic acid (3:4:3:0.2) as mobile phase revealed 6 spots in UV 254nm at Rf 0.03 (Light green), Rf 0.07 (Light Green), Rf 0.39 (Green), Rf 0.59 (Light green), Rf 0.74 (Green) and Rf 0.91 (Dark Green) (Figure 4).

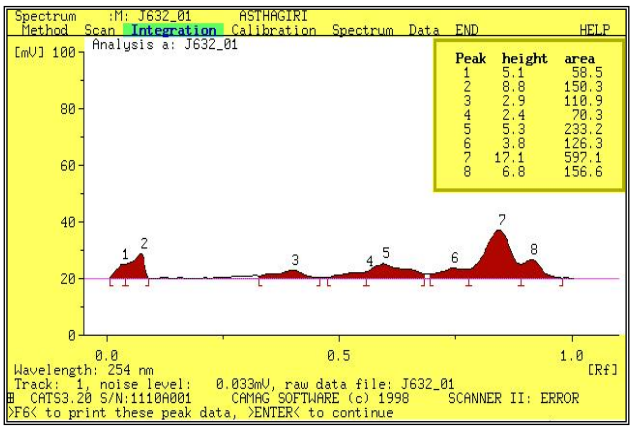

Figure 3: HPTLC Finger Prints of the extract of *E. hirta*

Figure 4: TLC Photo for *E. hirta*

FT-IR analysis of the *E. hirta* extract

The FT-IR spectrum of the methanolic extract of *Euphorbia hirta* exhibited distinct absorption bands corresponding to various bioactive functional groups. A broad and intense peak observed at 3995 cm^{-1} was attributed to hydrogen-bonded O–H stretching vibrations, indicating the presence of phenolic compounds and alcohol groups (Figure 5). The additional absorption band at 2680 cm^{-1} further confirmed O–H stretching, which is commonly associated with hydroxyl-containing secondary metabolites such as flavonoids and tannins. The absorption bands in the region of 1472–1402 cm^{-1} correspond to C–H bending vibrations characteristic of aliphatic alkane groups. A prominent peak at 1629 cm^{-1} was assigned to C=O (carbonyl) and C=N (imine) stretching vibrations, suggesting the presence of carbonyl-containing compounds such as flavonoids, phenolic acids, and other oxygenated phytoconstituents. The detection of these functional groups supports the presence of phenolic and bioactive secondary metabolites, which may be responsible for the observed antioxidant and cytotoxic activities of *Euphorbia hirta* extract.

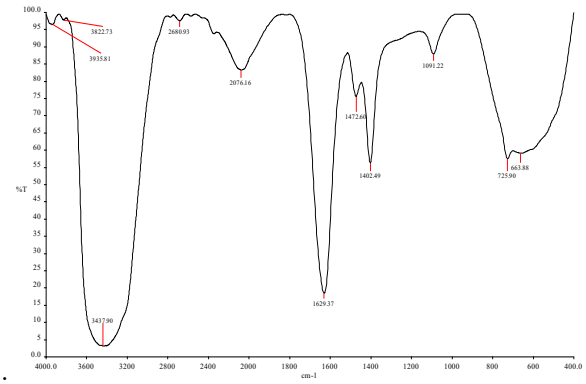

Figure 5: FT-IR analysis of the *E. hirta* methanolic extract

Anticancer activity (MTT assay)

The cytotoxic effect of the methanolic extract of *Euphorbia hirta* was evaluated at different concentrations ranging from 7.8 to 1000 µg/mL. The results demonstrated a concentration-dependent decrease in cell viability. At the highest concentration tested (1000 µg/mL), the extract exhibited strong cytotoxic activity with only 7.5% cell viability. As the concentration decreased, cell viability gradually increased, reaching 92.4% at the lowest concentration (7.8 µg/mL). The cell control group showed 100% viability with an absorbance value of 0.53 (Figure 6). The calculated IC_{50} value was approximately 108 µg/mL, indicating moderate cytotoxic potential of the extract.

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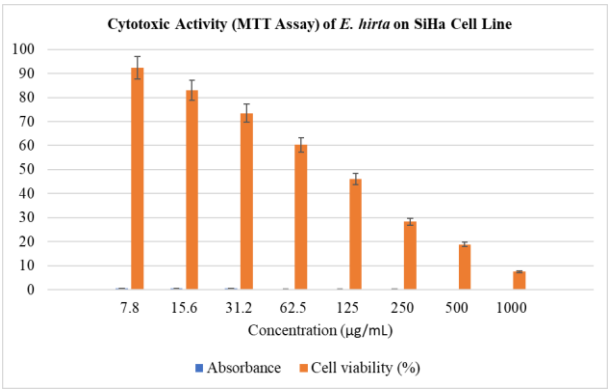

Figure 6: MTT assay of *E. hirta* on the SiHa cell line

Discussion

Medicinal plants remain integral to traditional healthcare systems and are increasingly recognized as valuable sources of structurally diverse bioactive compounds. Growing scientific interest has focused on systematically evaluating their phytochemical composition and biological activities, particularly antioxidant and anticancer potential. Such investigations not only validate traditional medicinal claims but also contribute to the identification of novel lead compounds for therapeutic development (Ojediran *et al*., 2024).

Among these medicinal species, *Euphorbia hirta* L. has attracted considerable attention due to its extensive ethnomedicinal applications and diverse pharmacological properties. Despite numerous reports highlighting its therapeutic potential, comprehensive studies integrating phytochemical profiling with antioxidant and cytotoxic evaluation using advanced analytical techniques remain limited. Therefore, the present study aimed to investigate the phytochemical composition, assess the antioxidant and cytotoxic activities, and identify major bioactive constituents of the methanolic extract of *Euphorbia hirta* using GC–MS and FT-IR analysis.

In the present study, preliminary phytochemical screening of the methanolic extract of *Euphorbia hirta* revealed the presence of tannins, alkaloids, saponins, flavonoids, terpenes, anthraquinones, and steroids. These findings are consistent with previous reports demonstrating the occurrence of diverse secondary metabolites in *E. hirta*. In similar studies, Venkatajothi Ramarao et al. (2023) reported that preliminary phytochemical screening of *Euphorbia hirta* revealed the presence of diverse secondary metabolites in the methanolic extract, including flavonoids, saponins, terpenoids, alkaloids, tannins, anthraquinones, steroids, and glycosides. Devsharma *et al*. (2023) reported that extracts prepared using different solvents such as ethanol, acetone, methanol, and water contained flavonoids, phenolics, and tannins; however, certain metabolites, including anthraquinones, reducing sugars, saponins, steroids, and terpenoids, were not detected in their study. Similarly, Panzu *et al*. (2020) documented the presence of alkaloids, flavonoids, tannins, steroids, and phenolic compounds in *E. hirta*. Al-Snafi (2017) further reported that aqueous, methanolic, and ethanolic extracts exhibited phenols, tannins, and quinones. The analysis of methanol extract revealed the moderate presence of steroids, terpenoids, alkaloids, carbohydrates, flavonoids, with traces of tannin, coumarins, cardiac glycosides, and phenols. Thus, the ethyl acetate-mediated extract of *Euphorbia hirta* was found to have more bioactive compounds compared with aqueous and methanol extracts (Sudhan *et al*., 2021).

In the present study, the methanolic extract of *Euphorbia hirta* demonstrated significant antioxidant activity in both DPPH radical scavenging and reducing power assays, exhibiting concentration-dependent effects in each model. The extract showed strong free radical scavenging ability with a low IC_{50} value in the DPPH assay and considerable electron-donating capacity in the ferric reducing assay, comparable to ascorbic acid. These findings indicate that the antioxidant potential of *E. hirta* may be attributed to its rich content of phenolic and flavonoid compounds, which act through hydrogen atom transfer and electron transfer mechanisms to neutralize reactive species. Comparatively, Sudhan *et al*. (2021) reported higher IC_{50} values for ethyl acetate (47.8 µg/mL), methanol (63.7 µg/mL), and aqueous extracts (181.86 µg/mL), suggesting comparatively lower antioxidant activity in those studies.

In the present study, GC–MS analysis of the methanolic extract of *Euphorbia hirta* revealed a wide range of phytochemical constituents, including ketones, phenolic derivatives, bicyclic compounds, fatty acid derivatives, and other secondary metabolites. The dominance of compounds at specific retention times indicates the substantial presence of bioactive constituents, which may be responsible for the observed antioxidant and cytotoxic effects. In similar studies,

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Ojediran *et al*. (2024) identified vinyl laurate, monoethylhexyl phthalate, and lauric acid triglyceride as the major compounds in the methanolic extract of *E. hirta*. Similarly, Karki *et al*. (2020) reported the identification of fifteen compounds in the methanol extract, with glycolaldehyde dimer being the principal constituent. In a related study, Velmurugan *et al*. (2025) found that 80 compounds were found using GC–MS analysis of the ethanol leaf extract of Euphorbia hirta, with lipid and lipid-like molecules making up the majority of these compounds (54%). Terpenoids (23%), fatty acyls (18%), monoradylglycerols (3%), steroid and steroid derivatives (9%), and quinone/hydroquinone lipids (1%) were among these; minor amounts of carboxylic acids, benzenoids, organic oxygen compounds, and heterocyclic compounds were also found. Terpenoids predominate among the lipid fractions, indicating that they constitute a significant bioactive class in the ethanol extract and may be responsible for the plant's pharmacological and anti-inflammatory properties.

In the present investigation, HPTLC analysis of the methanolic extract of *Euphorbia hirta* exhibited several well-resolved bands, with a dominant peak observed at Rf 0.84, suggesting the presence of a major phytoconstituent. Comparable findings were reported by Hazra *et al*. (2019), where optimized HPTLC conditions using silica gel 60 F_{254} plates and a mobile phase comprising hexane: ethyl acetate:acetone:1,4-dioxane: formic acid (4:3:2:1:0.5, v/v) resulted in effective separation, revealing multiple bands under UV (254 and 366 nm) and after derivatization, thereby confirming the phytochemical complexity of the extract.

In this study, the FT-IR spectrum of the methanolic extract of *Euphorbia hirta* revealed characteristic peaks corresponding to O–H, C–H, and C=O/C=N functional groups, indicating the presence of phenolic, flavonoid, and other oxygenated phytoconstituents. In similar studies, FT-IR analysis of the methanolic extract of *Euphorbia hirta* revealed characteristic C–H, CH_2, C–O (ester), and C=O (carboxylic) functional groups, indicating the presence of aliphatic and oxygenated phytoconstituents (Ojediran *et al*., 2024). Saravanakumar *et al*. (2022) reported that the FT-IR analysis of *Euphorbia hirta* extracts revealed the carbonyl, carboxyl, alkene, and hydroxyl functional groups in Ethiopian samples, while Indian samples exhibited characteristic aldehyde and carbonyl peaks, confirming the presence of diverse oxygenated phytoconstituents. FT-IR analysis revealed broad O–H stretching bands (3240–3294 cm^{-1}), saturated C–H stretching (2854–2924 cm^{-1}), C=O functional groups (1705 cm^{-1}), aromatic C=C vibrations (1604–1674 cm^{-1}), and NO_2-associated bands around 1500 cm^{-1}, confirming the presence of hydroxyl, carbonyl, aromatic, and nitro-containing phytoconstituents (Karki *et al*., 2020). Venkatesh *et al.* (2025) verified that *Euphorbia hirta* contains phenolic and flavonoid functional groups in addition to successfully forming nanoparticles. Their research also showed that *E. hirta* macromolecules have several binding interactions, indicating that a variety of phytoconstituents contribute to stability and biological activity.

In this study, the methanolic extract of *Euphorbia hirta* exhibited concentration-dependent cytotoxic activity (7.8–1000 µg/mL), reducing cell viability from 92.4% to 7.5%, with an IC_{50} value of approximately 108 µg/mL. These findings indicate moderate cytotoxic potential compared to the untreated control (100% viability). In similar studies, the anticancer potential of the ethyl acetate extract of *E. hirta* was evaluated using the sulforhodamine B (SRB) in vitro cytotoxicity assay against two cancer cell lines, namely lung cancer (NCI-H460) and liver cancer (Hep G2) cells. The ethyl acetate extract (EH-EA) at a concentration of 100 µg/mL demonstrated the most significant growth inhibitory effect on both NCI-H460 and Hep G2 cells compared to the other extract (Tran *et al*., 2020). Anitha *et al*. (2014) revealed that *Euphorbia hirta* leaf extract exhibited stronger cytotoxicity against Ehrlich Ascites Carcinoma (EAC) cells (59.67%) compared to Dalton Lymphoma Ascites (DLA) cells, demonstrating dose-dependent inhibition of proliferation and apoptosis induction. The ethanolic extract showed IC_{50} values of 384.7 µg/mL (EAC) and 560.83 µg/mL (DLA), indicating notable anticancer potential. *Euphorbia hirta* significantly inhibited MCF-7 cell survival (IC_{50} = 25.26 mg/mL at 24 h), inducing apoptosis characterized by DNA fragmentation, S and G2/M phase arrest, and activation of caspase-2, 6, 8, and 9 via a caspase-3-independent pathway. Among the fractions, the hexane fraction (HFsub4) exhibited the highest cytotoxic activity with an IC_{50} of 10.01 mg/mL (Kwan *et al*., 2016). According to Kalaivani *et al*. (2025), the methanolic

extract of *Euphorbia hirta* significantly reduced the growth of liver cancer cells by inducing apoptosis. Their investigation, which included flow cytometry, the MTT assay, and caspase-3 activity analysis to show a dose-dependent decrease in HepG2 cell viability, supported the idea that *E. hirta* is a viable natural option for the creation of new anticancer treatments that target liver cancers.

Conclusion

The present study demonstrates that the methanolic extract of *Euphorbia hirta* is rich in diverse bioactive secondary metabolites and exhibits significant antioxidant and moderate cytotoxic activities. The combined phytochemical screening, GC–MS, HPTLC, and FT-IR analyses confirmed the presence of phenolic, flavonoid, and other oxygenated compounds that may contribute to its biological effects. The observed cytotoxic activity against the SiHa cervical cancer cell line further supports its potential anticancer properties. Overall, these findings provide scientific validation for the traditional use of *Euphorbia hirta* and suggest that it may serve as a promising candidate for further pharmacological investigations and isolation of novel therapeutic agents.

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