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RESEARCH ARTICLE

Phytochemical profiling and selective cytotoxic activity of the hexane fraction of *Stellaria media* (L.) Vill. against human prostate cancer cells

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Abstract

Stellaria media (L.) Vill. is a medicinal plant traditionally used for various therapeutic purposes such as treatment of skin disorders, inflammatory conditions, digestive ailments and minor wounds; however, its anticancer potential against prostate cancer (PCa) has not been adequately investigated. This study aimed to characterise the phytochemical composition of the n-hexane fraction (F1) obtained from the whole plant of *S. media* and to evaluate its selective cytotoxic activity against human PCa cells. The n-hexane fraction (F1) was obtained by cold maceration of the dried whole plant and subjected to phytochemical profiling using gas chromatography–mass spectrometry (GC-MS). Cytotoxic activity was evaluated using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay against the human PCa cell line PC3 at different concentrations and incubation periods (24, 48 and 72 hr), while human dermal fibroblast cells (HdFn) were used to assess selectivity. The IC_{50} values were calculated accordingly. Gas chromatography–mass spectrometry (GC-MS) analysis revealed the presence of various non-polar phytochemical constituents, such as n-hexadecanoic acid, octacosanol and lanosterol. The n-hexane fraction exhibited significant cytotoxic activity against PC3 cells in a concentration- and time-dependent manner, with a progressive decrease in IC_{50} values over time. In contrast, higher IC_{50} values were observed in HdFn cells, indicating selective cytotoxicity toward cancer cells. Morphological observations further supported cytotoxic and apoptosis-related changes in treated PC3 cells. The n-hexane fraction of *S. media* demonstrated selective cytotoxic activity against human PCa cells, which may be attributed to identified non-polar phytochemical constituents. These findings suggest that *S. media* represents a promising source of plant-derived compounds for further anticancer investigations.

Keywords: cytotoxicity; gas chromatography-mass spectrometry analysis; hexane fraction; MTT assay; prostate cancer; *Stellaria media*

Introduction

Prostate cancer (PCa) is the second most diagnosed cancer in men worldwide and the fifth leading cause of cancer-related mortality, with an estimated 375000 deaths globally reported in 2020. The incidence of PCa increases markedly after the age of 50, establishing age as a major non-modifiable risk factor. In addition to aging, genetic predisposition, family history, lifestyle factors, dietary habits, metabolic disorders and chronic inflammatory conditions have been implicated in the initiation and progression of PCa (1–3).

Despite advances in screening and therapeutic strategies, the clinical management of PCa continues to face significant challenges, largely due to tumor heterogeneity, variable therapeutic responses and adverse effects associated with long-term treatment. Conventional treatment approaches, including chemotherapy, radiotherapy and hormone-based therapies, often lack adequate selectivity toward malignant cells, resulting in damage to normal tissues and systemic toxicity, which can negatively affect treatment efficacy and patient quality of life. These limitations have stimulated increasing interest in the identification of alternative anticancer agents with improved safety and selectivity profiles (4–6).

Medicinal plants represent an important source of structurally diverse bioactive compounds and have attracted considerable scientific interest in cancer research. Among plant-derived constituents, non-polar and semi-polar phytochemicals have received particular attention due to their potential cytotoxic and anticancer properties. Lipophilic compounds such as fatty acids, terpenoids, sterols and related hydrophobic molecules are known to modulate cellular membrane integrity, mitochondrial function and intracellular signalling pathways involved in cell proliferation and apoptosis (7–9).

Stellaria media (L.) Vill., commonly known as chickweed, is an annual herbaceous plant belonging to the Caryophyllaceae family, characterised by slender stems, small oval leaves and tiny white star-shaped flowers, widely distributed across temperate regions of Europe, Asia and the Middle East. The plant has a long history of use in traditional medicine for the treatment of skin disorders, inflammatory conditions, digestive ailments and minor wounds. In several regions, *S. media* is also consumed as leafy vegetables, suggesting acceptable safety and tolerability during prolonged use. This traditional background has encouraged

scientific investigation into the phytochemical composition and biological activities of the plant (10–13).

Phytochemical investigations have shown that *S. media* contains a variety of bioactive constituents, including fatty acids, sterol-related compounds, terpenoid derivatives, flavonoids, saponins and other secondary metabolites that may collectively contribute to its reported pharmacological activities. These constituents have been associated with antioxidant, anti-inflammatory, antibacterial and metabolic regulatory effects. Recent studies have reported cytotoxic effects of *S. media* extracts against different cancer cell lines, suggesting potential anticancer activity mediated through mechanisms such as mitochondrial dysfunction, oxidative stress modulation and apoptosis induction (14–16).

However, evidence remains limited regarding the activity of chemically defined fractions of *S. media*, particularly against PCa cells and the relationship between phytochemical composition and observed cytotoxic effects has not been adequately established. A major limitation of previous investigations is their reliance on crude extracts, which hampers clear attribution of biological activity to specific classes of compounds (17, 18).

In this context, gas chromatography-mass spectrometry (GC-MS) is a well-established analytical technique for profiling non-polar phytochemicals, including fatty acids, terpenoids and sterol-related compounds, many of which have been associated with anticancer mechanisms such as apoptosis induction and oxidative stress regulation (19, 20).

Despite growing interest in *S. media*, a clear research gap persists regarding the cytotoxic potential of its non-polar fractions against PCa cells and the phytochemical basis underlying such effects (21, 22). Addressing this gap is essential for improving the pharmacological understanding of *S. media* and evaluating its potential as a source of plant-derived anticancer agents as well as to the best of our knowledge, this is the first study to correlate the cytotoxic activity of a non-polar n-hexane fraction of *S. media* with its GC-MS phytochemical profile against PCa cells and normal human dermal fibroblast cells used to assess selectivity.

Materials and Methods

Apparatus and instruments

The following instruments and laboratory equipment were used: an analytical balance was used for accurate weighing of plant material (Sartorius, Germany); a rotary evaporator connected to a vacuum pump was used for solvent removal and extract concentration (Büchi, Germany); standard glassware including beakers, graduated cylinders, conical flasks and glass funnels was used. Filter paper was employed for filtration processes. Gas chromatography–mass spectrometry analysis was performed using a GC–MS system (Agilent Technologies 7820A gas chromatograph coupled with a 5977E mass selective detector, USA). Separation was achieved on an Agilent HP-5ms Ultra Inert capillary column (30 m × 250 µm i.d. × 0.25 µm film thickness).

Cell culture plates (96-well) and flasks were used for pharmacological assays. A CO_2 incubator was used to maintain cell cultures and a laminar flow hood was used to ensure sterile working conditions. An autoclave, Millipore filter (0.22 µm), hemocytometer, micropipettes for precise liquid handling, an inverted light microscope for morphological observations and a microplate reader (ELISA, 575 nm) were utilised for absorbance measurements in cytotoxicity assays.

Chemicals and reagents

The n-hexane was used for maceration. The identification of chemical constituents was achieved by comparing the obtained mass spectra with those in the NIST11 Mass Spectral Library (C:\GCMS\firmware\NIST11.L), deionized / distilled water was used for solution preparation and washing. Helium (99.99 % purity) was used as the carrier gas at a constant pressure of 11.933 psi with an injection volume of 1 µL in splitless mode. preparation and washing. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT kit) was purchased from Intron Biotech (Korea). The RPMI-1640 medium containing HEPES, L-glutamine, sodium bicarbonate, penicillin, streptomycin and 15 % fetal bovine serum was used for culturing the PC3 and HdFn cell lines. Phosphate buffered saline (PBS), trypsin solution (1 %), ethylenediaminetetraacetic acid (EDTA) solution (1 %) and trypsin-EDTA mixture were prepared in the laboratory. Fetal bovine serum (FBS), antibiotics (penicillin and streptomycin) and sodium bicarbonate were added to the medium as required. All chemicals and reagents used were of analytical grade and distilled water was used for solution preparation.

Plant material collection

Stellaria media specimens were collected from the high-altitude regions of Sulaymaniyah (northern Iraq) between March and May (2025). This period was selected due to moderate temperatures and adequate humidity, during which the plant exhibits vigorous growth and a high content of bioactive constituents. The plant was authenticated by Prof. Dr. Sukaena Abbas Ealewy, Department of Biology, College of Science, University of Baghdad. Collected plant material was cleaned thoroughly to remove foreign matter, was dried in a well-ventilated shaded area and was powdered into a fine powder using an electric grinder.

Preparation of *Stellaria media* extract

Extraction was performed according to standard extraction procedures. A total of 200 g of dried whole plant material was subjected to cold maceration with n-hexane for 72 hr. The extract was filtered every 24 hr and the solvent was replaced with a fresh volume. After completion of the extraction period, the combined filtrates were concentrated under reduced pressure using a rotary evaporator (23). The obtained n-hexane extract (F1) was divided into 2 portions: one portion was used for GC-MS analysis and the other was used for cytotoxic evaluation.

Gas chromatography–mass spectrometry analysis

The n-hexane extract of *S. media* plant was analysed to identify the bioactive components using gas chromatography system employing the following condition: Phytochemical analysis was carried out using a GC-MS system (Agilent Technologies 7820 A gas chromatograph coupled with a 5977 E mass selective detector, USA). Separation was achieved on an Agilent HP-5 ms Ultra Inert capillary column (30 m × 250 µm i.d. × 0.25 µm film thickness). Helium (99.99 % purity) was used as the carrier gas at a constant pressure of 11.933 psi with an injection volume of 1 µL in splitless mode. The injector and auxiliary heater temperatures were set at 250 °C and 310 °C, respectively. The oven temperature program was initiated at 60 °C (held for 3 min), increased to 180 °C at 6 °C/min and then raised to 280 °C at 7 °C/min and held for 10 min. Mass spectra were obtained using electron ionisation at 70 eV over a scan range of m/z 25–1000. The ion source

and quadrupole temperatures were maintained at 230 °C and 150 °C, respectively (24).

Cytotoxicity testing

The methodology described here aims to investigate the effects of n-hexane extract (fraction F1) on the viability of the human PCa cell line (PC3), as well as its effect on normal human dermal fibroblast cells (HdFn), using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay. This assay is widely used to assess cell metabolic activity, which serves as an indicator of cell viability, proliferation and cytotoxicity. Living cells with active oxidoreductase enzymes can reduce the yellow MTT reagent to insoluble purple formazan crystals (25). The quantity of formazan formed is directly proportional to the number of viable cells present. Thus, by measuring the absorbance of dissolved formazan, the cytotoxic effects of the *S. media* n-hexane extract (F1) were quantitatively evaluated on both cancer and normal cell lines.

Cell culture

The human PCa cell line (PC3), originally established in the United States (USA) from a human PCa bone metastasis and normal human dermal fibroblast cells (HdFn) were obtained from the tissue culture laboratory. The PC3 cells were used at passage number 11. Both cell lines were maintained as monolayers in RPMI-1640 medium supplemented with 15 % fetal bovine serum (FBS), 1 % penicillin (10^3 IU), 0.001 g streptomycin, 1 % sodium bicarbonate and L-glutamine and HEPES as provided by the manufacturer. Cells were incubated in a humidified atmosphere containing 5 % CO_2 at 37 °C. Subculturing was performed when the cells reached 70–80 % confluency. The growth medium was removed and cells were washed twice with phosphate buffered saline (PBS). Trypsin-EDTA solution was added and plates were incubated at 37 °C for approximately 2 min until cells detached. The reaction was stopped by adding complete RPMI-1640 medium and cells were resuspended and seeded as required. Cell counts were performed using a hemocytometer to ensure the desired seeding density.

Cell viability assay

The cytotoxic effect of the n-hexane extract of *S. media* (F1) was evaluated using the MTT assay. The PC3 and HdFn cells were seeded into 96-well plates (200 µL per well) and were allowed to attach overnight. After 24 hr, the culture medium was replaced with fresh medium containing serial dilutions of the F1 extract (25, 50, 100, 200 and 400 µg/mL). Each concentration, including control, was assayed in triplicate. Cells were incubated at 37 °C in a 5 % CO_2 incubator for 24, 48 and 72 hr. Following incubation, 10 µL of MTT solution was added to each well and plates were incubated for an additional 4 hr in the dark. The medium was then gently removed and replaced with 100 µL of solubilisation solution and plates were incubated for 5 min. The absorbance was measured using a microplate reader at 575 nm. Cell viability (%) for each sample was calculated as:

Cell viability (%) = (Absorbance of treated cells/absorbance of control cells) × 100.

Morphological evaluation

Morphological evaluation of PC3 cells was performed under an inverted microscope at 40x magnification to observe apoptosis-related changes such as cell shrinkage and detachment. Images were captured before and after treatment with F1 extract for up to 72 hr. to document morphological alterations associated with cytotoxicity.

Statistical analysis

All MTT assay results were represented as mean ± standard deviation (SD) from 3 independent experiments. The IC_{50} (the concentration at which 50 % cell viability is inhibited) was calculated using non-linear regression analysis in GraphPad Prism 9.2. One-way ANOVA was used to compare differences among treated groups. A *p*-value less than 0.05 was considered statistically significant.

Results

Extraction of Iraqi *Stellaria media* plant

The extraction of *S. media* using maceration with n-hexane resulted in a measurable yield of non-polar phytochemicals. From an initial plant weight of 200 g, the extraction process yielded a hexane-soluble portion of 3.41 g, corresponding to a yield of 1.71 % (w/w). This extract was selected for subsequent GC-MS characterisation and cytotoxic evaluation.

Gas chromatography-mass spectrometry (GC-MS) analysis

Gas chromatography-mass spectrometry analysis was performed on the n-hexane fraction of *S. media* to identify the major non-polar bioactive phytochemical constituents. The analysis revealed the presence of several lipophilic compounds, of which 12 major constituents were selected based on their peak number, molecular weight, retention time, similarity index, chemical class and reported pharmacological activity. The GC-MS chromatogram of the n-hexane fraction was presented in Fig. 1, while the peak numbers, compound names, molecular weights, retention times, chemical classes, similarity index and reported pharmacological activities of the identified compounds were summarised in Table 1 according to their elution order from the HP-5ms capillary column.

Cytotoxicity testing

The cytotoxic activity of the n- hexane fraction (F1) obtained from *S. media* was evaluated against the human PCa cell line (PC3) using the MTT assay. Human dermal fibroblast cells (HdFn) were used as a normal cell line to assess the selectivity of the tested fraction. The assay was performed at five different concentrations (25, 50, 100, 200 and 400 µg/mL) and 3 incubation periods (24, 48 and 72 hr). Cell viability (%) data for PC3 and HdFn cells were summarised in Tables 2 and 3, while the corresponding graphical cytotoxic profiles and IC_{50} values were illustrated in Fig. 2–4.

After 24 hr of exposure, fraction F1 exhibited a concentration-dependent reduction in PC3 cell viability. The IC_{50} value for PC3 cells was calculated as 72.04 µg/mL, whereas a higher IC_{50} value of 117.6 µg/mL was observed for HdFn cells.

At 48 hr, the cytotoxic effect of fraction F1 was markedly enhanced against PC3 cells, as reflected by a decrease in the IC_{50} value to 59.95 µg/mL, while the IC_{50} for HdFn cells remained comparatively higher (111.8 µg/mL).

Following 72 hr of treatment, fraction F1 demonstrated the strongest cytotoxic activity against PC3 cells, with an IC_{50} value of 49.12 µg/mL, compared to 78.03 µg/mL for HdFn cells.

Morphological alterations of PC3 cell line after exposure to F1 fraction

As shown in Fig. 5, exposure of the human PCa cell line (PC3) to the n-hexane fraction (F1) of *S. media* resulted in a marked reduction in

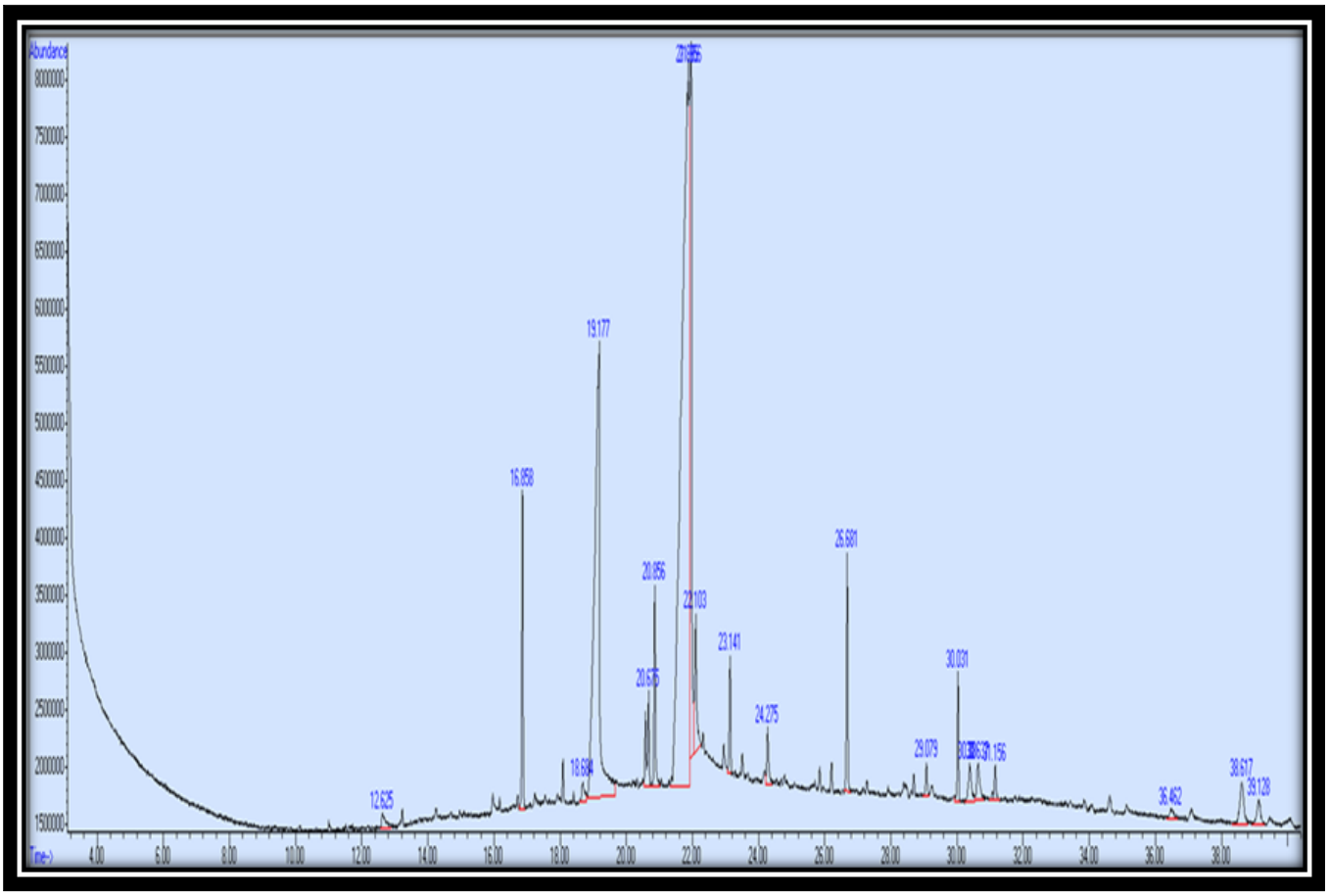

Fig. 1. Gas chromatography-mass spectrometry chromatogram of the n-hexane fraction of Iraqi *Stellaria media*.

Table 1. Major compounds identified in the n-hexane fraction (F1) of Iraqi *Stellaria media* by gas chromatography-mass spectrometry analysis

Peak No.	Compound name with molecular weight	Retention time in Min.	Class of compounds	Similarity index %	Pharmacological activity
4	n-hexadecanoic acid (palmitic acid) (256.42)	20.28	Saturated fatty acid	98	Cytotoxic, antioxidant and apoptosis induction
5	9,12,15-Octadecatrienoic acid, methyl ester (Z,Z,Z) (Methyl linolenate) (294.48)	20.68	Fatty acid ester	92	Antioxidant and lipid peroxidation inhibitor.
6	Phytol acetate (338.53)	20.86	Diterpene Ester	96	Cytotoxic, antioxidant and ROS-mediated apoptosis.
7	9,12-Octadecadienoic acid (Z,Z) (linoleic acid) (280.45)	21.87	Unsaturated fatty acid	98	Anti-inflammatory, antioxidant and anticancer
8	9,12,15-Octadecatrienoic acid (Z,Z,Z) (α-Linolenic acid) (278.43)	21.95	Polyunsaturated fatty acid	99	Antioxidant, cardio protective and anti-inflammatory
9	Octadecanoic acid (Stearic acid) (284.48)	22.10	Saturated fatty acid	96	Antimicrobial and antioxidant
10	9-Tricosene (Z) (322.62)	23.14	Alkene hydrocarbon	99	Antioxidant, antimicrobial and insecticidal activity
14	Squalene (410.72)	30.03	Triterpene hydrocarbon	99	Antioxidant, chemo preventive and immune modulatory
17	Octacosanol (410.77)	31.15	Long-chain alcohol	93	Antioxidant and cytoprotective
18	β-Sitosterol (414.71)	36.46	Phytosterols	95	Anti-inflammatory, induce apoptosis, anticancer and cholesterol-lowering
19	γ-sitosterol (414.71)	38.62	Phytosterols	95	Anti-inflammatory, induce apoptosis and anticancer, cholesterol-lowering
20	Lanosterol (426.73)	39.12	Triterpenoid	95	Anti-inflammatory, cytotoxic and precursor of steroids

Table 2. Cell viability (%) of PC3 and HdFn cells after 24 and 48 hr of exposure to the n-hexane fraction (F1) of *Stellaria media*

Concentration (µg/mL)	Cell viability (%) for 24 hr		Cell viability (%) for 48 hr	
	F1		F1	
	HdFn	PC3	HdFn	PC3
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
400	69.984±0.406	40.220±1.042	67.557±1.257	36.886±1.095
200	75.772±0.698	46.685±0.899	73.278±0.841	42.361±0.947
100	84.876±0.637	55.552±0.848	82.848±0.633	52.431±2.554
50	94.637±0.177	70.029±0.358	92.830±1.003	64.425±0.566
25	96.065±0.645	79.526±0.654	95.696±0.662	75.866±0.743

Table 3. Cell viability (%) of PC3 and HdFn cells following 72 hr treatment with the n-hexane fraction (F1) of *Stellaria media*

Concentration (µg/mL)	Cell viability (%) for 72hr	
	F1	
	HdFn	PC3
	Mean ± SD	Mean ± SD
400	65.498±0.421	28.782±0.241
200	72.839±1.457	36.021±0.749
100	78.164±0.813	41.059±0.564
50	87.616±1.514	58.176±0.371
25	93.711±0.241	71.867±0.779

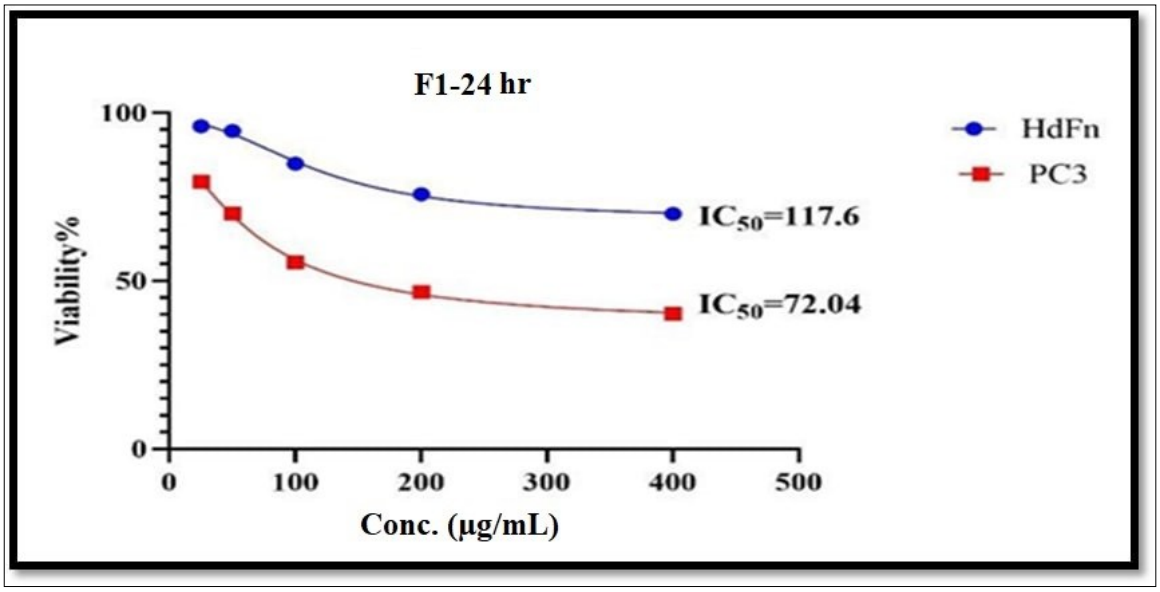

Fig. 2. Cytotoxic activity of F1 in PC3 and HDFn cells after 24 hr of treatment.

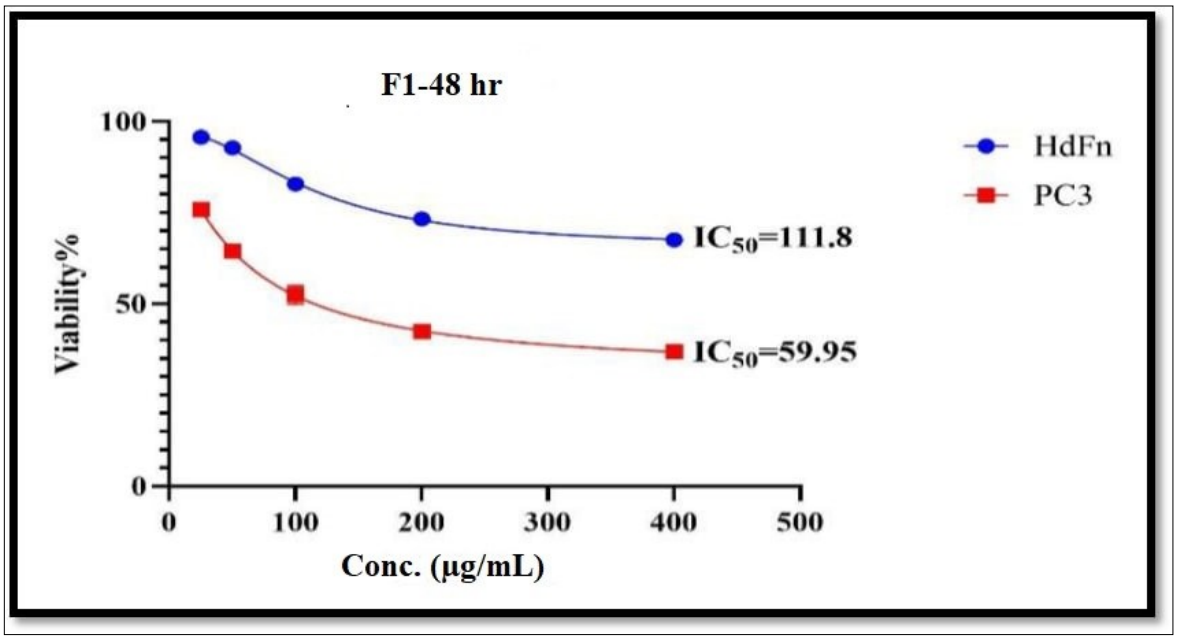

Fig. 3. Cytotoxic activity of F1 in PC3 and HDFn cells after 48 hr of treatment.

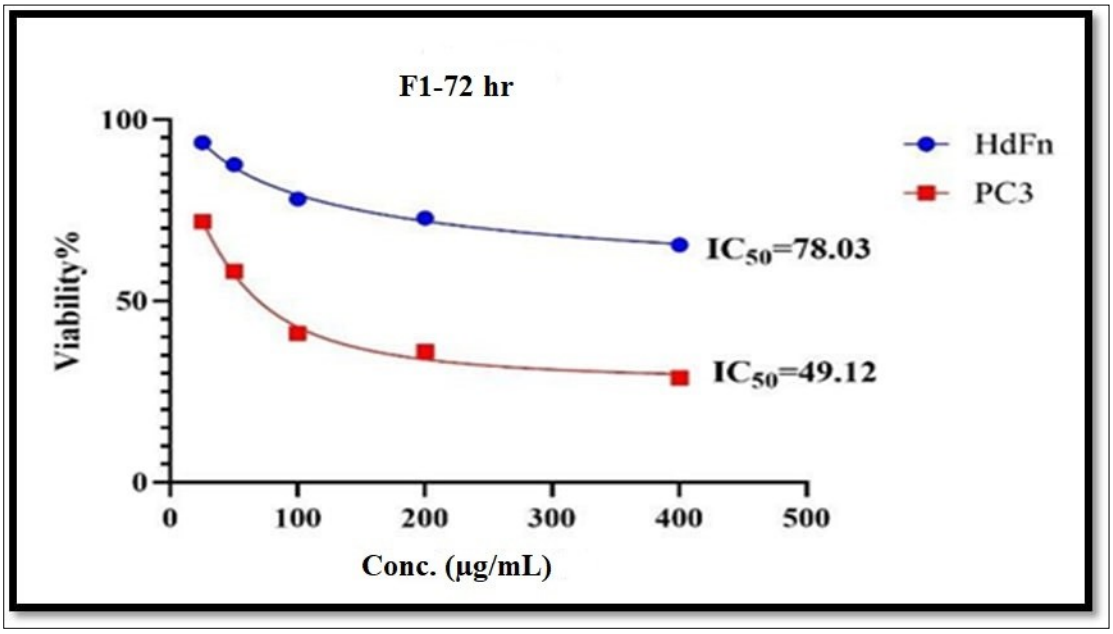

Fig. 4. Cytotoxic activity of F1 on PC3 and HdFn cells after 72 hr of treatment.

the number of viable cancer cells, accompanied by pronounced morphological alterations. These changes included cell shrinkage, loss of normal cell architecture, cell detachment from the culture surface and increased cell separation, which are characteristic features of cytotoxic and apoptotic cell death.

Discussion

The extraction method represents a critical step in phytochemical investigations, as it directly influences the chemical composition, yield and biological activity of plant-derived extracts; therefore, cold maceration was employed to preserve thermolabile phytochemical constituents that may be degraded by heat (24). The n-Hexane was selected as the extraction solvent due to its high affinity for non-polar phytochemicals, enabling the selective enrichment of lipophilic constituents associated with cytotoxic and anticancer activities (26).

In line with the selection of cold maceration and n-hexane as an extraction solvent, GC-MS analysis of the n-hexane fraction revealed a diverse profile of lipophilic constituents, predominantly fatty acids and their esters, diterpene derivatives, long-chain aliphatic alcohols, triterpenes and phytosterols. Compound identification was achieved by comparison of the obtained mass spectra with reference spectra from the NIST mass spectral library (27). Some minor peaks remained unidentified due to the absence of matching reference spectra. The extraction yield supports previous findings that non-polar solvents efficiently extract fatty acids, sterols, terpenoids and other hydrophobic compounds from medicinal plants, many of which are associated with biological activity. For example, n-hexadecanoic acid (palmitic acid) has been reported to exhibit cytotoxic and antioxidant activities through modulation of pathways involved in cancer cell death (28). Methyl linolenate and other fatty acid esters are known for their antioxidant properties and their ability to inhibit lipid peroxidation, thereby influencing cellular redox balance (29). Phytol acetate, a diterpene derivative detected in the n-hexane fraction, has been reported to exert cytotoxic and antioxidant effects, primarily through reactive oxygen species generation and mitochondrial dysfunction (30). Linoleic acid and α-linolenic acid, two polyunsaturated fatty acids identified in the fraction, have been shown to inhibit cell proliferation by altering membrane lipid composition and modulating oxidative stress and inflammatory signaling pathways (31, 32). Stearic acid has also been

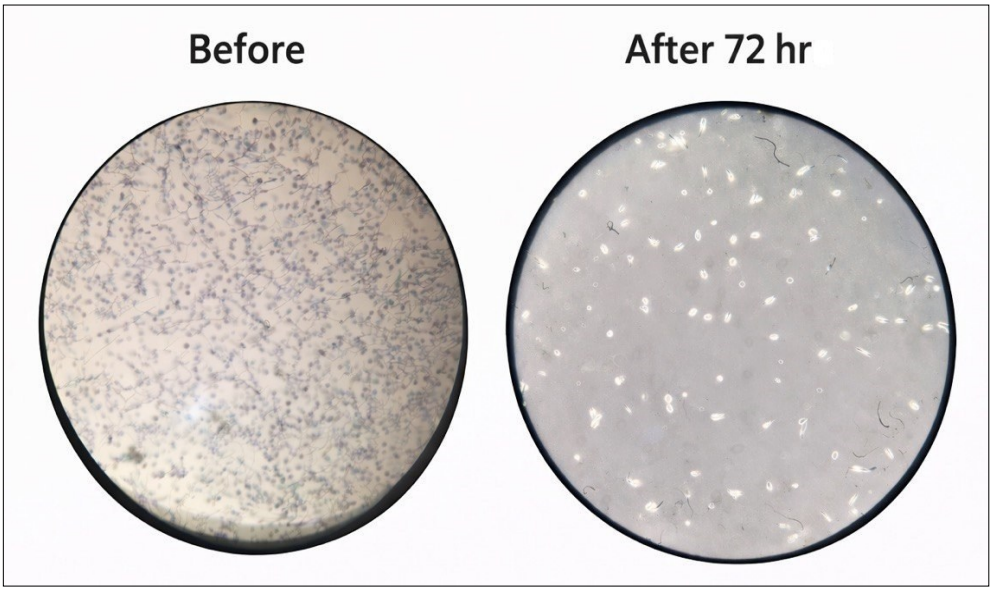

Fig. 5. Morphological alterations in PC3 cells following treatment with the n-hexane fraction (F1) of *Stellaria media*.

reported to stabilise cell membranes and reduce lipid peroxidation, contributing to its protective and antioxidant properties (33). The identification of 9-tricosene, an unsaturated hydrocarbon, supports the potential antibacterial and radical-scavenging activities of the n-hexane fraction (34). Squalene, a hydrocarbon structurally related to other non-polar constituents, is known for its ability to quench singlet oxygen and protect cells against oxidative stress (35). Additionally, octacosanol, a long-chain aliphatic alcohol, has been reported to possess cytoprotective, antioxidant and antiproliferative activities (36). The presence of phytosterols such as β-sitosterol and γ -sitosterol further enhances the pharmacological significance of the n-hexane fraction. These compounds have been shown to modulate cell cycle progression, inhibit cancer cell proliferation and induce apoptosis in various cancer models (37, 38). Moreover, the detection of lanosterol, a triterpenoid sterol, adds to the medicinal relevance of the extract, as this compound has been reported to exhibit cytotoxic and oxidative stress–regulating properties (39, 40).

Moreover, previous study has demonstrated a clear association between GC–MS–identified non-polar phytochemicals and cytotoxic activity, where lipophilic compounds detected by GC-MS profiling were directly correlated with antiproliferative and apoptosis-inducing effects across various cancer cell lines (24).

Following GC-MS characterisation of the n-hexane fraction, the cytotoxic potential of fraction F1 was evaluated using the MTT assay to correlate phytochemical composition with biological activity. The results demonstrated that the n-hexane fraction significantly inhibited the growth of human PCa (PC3) cells, while exhibiting comparatively lower toxicity toward normal human dermal fibroblast (HdFn) cells, indicating a selective cytotoxic effect. Fraction F1 reduced PC3 cell viability in both a concentration- and time-dependent manner, as evidenced by the progressive decrease in IC_{50} values from 24 to 72 hr. In contrast, HdFn cells consistently exhibited higher IC_{50} values, confirming that the cytotoxic activity of the fraction was more pronounced in cancer cells than in normal cells. Such selectivity represents an important criterion in the preliminary evaluation of plant-derived extracts for potential anticancer applications.

Morphological alterations observed in PC3 cells following treatment with the n-hexane fraction, including cell contraction, reduced adhesion, membrane irregularities and cell detachment, provide additional evidence supporting apoptosis-associated cell death and are consistent with the MTT assay findings.

Collectively, these results suggest that the cytotoxic activity of the n-hexane fraction of *S. media* arises from the synergistic effects of multiple non-polar phytochemicals identified in the extract. Such interactions may influence oxidative stress regulation, mitochondrial function and apoptosis-related signaling pathways, ultimately contributing to the inhibition of PCa cell growth.

The integration of phytochemical profiling with biological evaluation highlights the pharmacological potential of *S. media* as a promising source of plant-derived anticancer agents.

Conclusion

This study demonstrated that the n-hexane fraction (F1) of *Stellaria media* is rich in bioactive non-polar phytochemicals, as confirmed by GC-MS analysis. The identified compounds mainly included fatty acids, fatty acid esters, diterpene derivatives, triterpenes, long-chain aliphatic alcohols and phytosterols, which are recognised for their biological relevance. The cytotoxic evaluation revealed that fraction (F1) exerted a significant inhibitory effect on PC3 prostate cancer cells in a concentration- and time-dependent manner, while exhibiting lower toxicity toward normal human dermal fibroblast cells. This selective cytotoxic response highlights the potential of the n-hexane fraction as a promising source of plant-derived anticancer agents. Overall, the findings support the pharmacological potential of *S. media* and provide a scientific basis for further studies aimed at isolating active constituents and validating their anticancer efficacy through *in vivo* investigations.

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Authors' contributions

NDM participated in the study design, conducted the research and performed the statistical analysis. TZAJ designed the study, contributed to its sequencing and revised the drafted manuscript. Both authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Ethical issues: None

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