Article

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In Vitro Evaluation of Chemical Compounds, Antiproliferative Activity, Efficacy in Apoptosis Induction and Cell Cycle Arrest of *Heliotropium Bacciferum* Extract and Fractions on the A549 Cell Line

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Background: Medicinal plants and natural products have proven effective in inhibiting the growth of cancer cells due to their rich content of active compounds, with polyphenols being among the most prominent. This study aims to assess the antiproliferative activity of *Heliotropium bacciferum* extracts on breast (MCF-7) and lung (A549) cancer cell lines and identify the extract's key active compounds.

Methods: The antiproliferative activity of *Heliotropium bacciferum* extract and fractions on breast (MCF-7) and lung (A549) cancer cell lines was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Subsequently, the hexane extract was selected as the most effective extract for investigating the mechanism of its antiproliferative activity on A549 cell lines. The investigation involved the evaluation of cell cycle alterations using propidium iodide staining and the induction of apoptosis using the Annexin V-FITC/PI kit. Finally, gas chromatography/mass spectrometry (GC/MS) analysis was conducted to identify the chemical compounds in the hexane extract of *Heliotropium bacciferum*.

Results: According to the MTT assay, the hexane fraction of *Heliotropium bacciferum* effectively halted the growth of A549 and MCF-7 cancer cells with IC_{50} values of 104.14 and 83.84 µg/mL, respectively. Additionally, methyl lineoleate (36.5%), linoleic acid (19.1%), and 3′-Acetyllycopsamine (10.7%) were the major components in the *Heliotropium bacciferum* hexane fraction, according to GC/MS analysis.

Conclusions: In order to produce pharmaceutical anticancer medicines for cancer therapies, *Heliotropium bacciferum* hexane fraction can be employed.

Keywords: *Heliotropium bacciferum*; hexane fraction; cell cycle; apoptosis; MCF-7; A549

Introduction

Cancer remains one of the leading causes of death worldwide, ranking second as the most common cause of death after cardiovascular diseases [1,2]. According to data from the World Health Organization (WHO), nearly 10 million people succumb to cancer annually, equating to one in six deaths [3]. Among the most prevalent cancers, lung and breast cancers top the list, accounting for approximately 2.26 million new cases of lung cancer and nearly 2.21 million new cases of breast cancer. In terms of mortality, lung cancer ranks first with 1.80 million deaths, while breast cancer ranks fifth with 685,000 deaths [3].

Numerous methods are used to treat cancer, which can be applied individually or in combination, depending on the specific pathology. These treatment regimens include immunotherapy, hormone therapy, chemotherapy, hyperthermia, stem cell transplantation, photodynamic therapy, radiation therapy, surgery, and targeted therapy [4–6]. Despite the significance of these treatment approaches, they are often associated with undesirable side effects, and their efficacy may diminish with time. Furthermore, they can affect healthy tissues and organs [6]. Given these limitations, numerous studies in recent years have sought alternatives for cancer treatment. These alternatives aim to reduce the side effects associated with established cancer treatments or enhance their effectiveness [1,7,8]. Among these alternatives, natural products and medicinal plants have emerged as promising candidates due to their efficacy in treating various types of cancer. These natural products often contain active compounds such as polyphenols and flavonoids, which play pivotal role in suppressing carcinogenesis [9,10]. These compounds inhibit cancer cell growth through mechanisms that involve modifying signaling path-

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ways, inhibiting cell cycle events, regulating enzymes associated with cancer cell proliferation, and induction of apoptosis [7,10,11].

Heliotropium bacciferum (*H. bacciferum*) is a notable medicinal plant renowned for its rich content of active compounds. This species belongs to the genus *Heliotropium* and the Boraginaceae family, comprising over 100 genera and more than 2000 species [12]. In alternative medicine, *H. bacciferum* has been widely adopted. Various parts of *H. bacciferum* are used to treat skin conditions such as abscesses, boils, tinea, tonsillitis, and burns [13]. In Saudi Arabia, *H. bacciferum* is used to treat scorpion stings [14]. Recent studies have illuminated this species' biological properties, including antifungal, antibacterial, anticancer, anti-inflammatory, antidiabetic and anti-hyperlipidemia activities [13,15,16]. These beneficial effects are attributed to its active constituents, with pyrrolizidine alkaloids being one of the most significant [13,15]. A study conducted by Aïssaoui *et al*. [13] assessed the anticancer potential of *H. bacciferum* extracts on DLD1 and HCT116 cancer cell lines. The findings indicated that the methanol extract had no discernible effect on the cancer cell lines, while the chloroform extract exhibited activity at concentrations ranging from 62–95 µg/mL [13].

To our knowledge, the mechanistic underpinnings of the anticancer properties of *H. bacciferum* remain explored. Therefore, this study aimed to evaluate the antiproliferative activity of various extracts of *H. bacciferum* on MCF-7 and A549 cancer cell lines. Subsequently, the hexane extract was selected for cell cycle analysis and to investigate the induction of apoptosis in A549 cells. Additionally, the chemical compounds in hexane extract were determined using gas chromatography/mass spectrometry (GC/MS) analysis, shedding light on the potential mechanisms of action in the species responsible for inhibiting cancer cell growth.

Materials and Methods

Plant Source

Aerial parts of *H. bacciferum* were collected in March 2022 from Saudi Arabia. The plant was identified by a botanist and, a voucher specimen (HBQU-041511) was deposited at the herbarium of King Saud University.

Preparation of Plant Extract and Fractions

A Soxhlet device was employed to extract dried powder from the aerial parts of *H. bacciferum* using three liters of ethanol. After evaporating the liquid extract using a rotary evaporator, the extraction yielded approximately 45 g of raw methanolic extract. Subsequently, the raw extract was sequentially partitioned into different polar solvents (n-hexane, chloroform, and n-butanol) to obtain 2, 4, 13, and 17.5 g of dried fractions.

Antiproliferative Activity

Cell Culture

The breast cancer cell line MCF-7 (ACC 115) and the lung cancer cell line A549 (ACC 107) were provided by the German Collection of Microorganisms and Cell Cultures (DSMZ Leibniz Institute, Braunschweig, Germany). The cell lines were verified to be free of mycoplasma contamination, and their identity was confirmed via short tandem repeat (STR) profiling. The cells were incubated in Dulbecco's modified eagle's medium (DMEM-5671) containing 4.5 g/L of glucose, 10% fetal bovine serum (FBS), and 1% of the antibiotics (streptomycin, penicillin, glutamine, and non-essential amino acids).

Cytotoxicity Activity Using MTT Assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was utilized to assess cytotoxicity following a protocol by Nasr *et al*. [17]. The *H. bacciferum* crude extract and fractions were applied to MCF-7 and A549 cell lines at various concentrations for 48 hours, with untreated cells serving as the control. After treatment, cells were exposed to MTT for 2–4 hours, and the formazan product was dissolved in acidified isopropanol for 15 minutes. The absorbance at 570 nm was measured using a microplate reader. The IC_{50} (concentration that causes 50% inhibition) was determined to assess the cytotoxicity of the extract. Cell viability percentage was determined as follows:

$$\text{Cell viability (\%)} = \text{Mean absorbance of treated cells} / \text{mean absorbance of untreated cells (control)} \times 100$$

The IC_{50} values were calculated from the graph of cell viability percentage plotted against sample concentrations according to the following equation:

$$IC_{50} = (50 - b)/a$$

a and b: From the linear equation of the curve.

Cell Cycle Analysis

The impact of the *H. bacciferum* hexane fraction on A549 cell cycle distribution was determined by staining with propidium iodide (PI) [17,18]. Briefly, A549 cells were plated and cultured for 24 hours with or without the IC_{50} and 2 IC_{50} doses of the hexane fraction. Following treatments, cells were trypsinized, collected, and fixed in 70% ice-cold ethanol at 4 °C for 4 hours. Phosphate-buffered saline (PBS) was used to wash the cells. The cells were then centrifuged, rinsed with ice-cold PBS, and stained with 50 µg/mL propidium iodide while 100 µg/mL RNase was added for 30 min at 37 °C in the dark. Flow cytometry was used to identify cell cycle distribution (CytoFLEX S, Beckman Coulter, Brea, CA, USA).

Table 1. IC_{50} values for the *Heliotropium bacciferum* (*H. bacciferum*) extract and fractions.

Cell Lines	*H. bacciferum* extract and fractions IC_{50} (µg/mL)			
	Crude	Hexane	Chloroform	Butanol
A549	ND	83.84 ± 0.48	ND	ND
MCF-7	ND	104.14 ± 1.2	ND	ND

ND, Not Determined.

Apoptosis Quantification by Annexin V-FITC/PI

Apoptosis was investigated using the Annexin V-FITC/PI kit (BioLegend, San Diego, CA, USA) following the manufacturer's instructions. A549 cells were exposed to IC_{50} and 2 IC_{50} doses of the hexane fraction for 24 hours, along with the control group. Subsequently, detached and adherent cells were gathered and incubated for 15 min at room temperature with 5 µL of FITC conjugated with annexin V and 5 µL of PI in the dark. Using flow cytometry, live, early, late, and necrotic cells were quantified (Beckman Coulter, Brea, CA, USA).

Compounds Identification by GC/MS Analysis

The chemical components of the *H. bacciferum* hexane fraction were identified using gas chromatography/mass spectrometry (GC/MS) (Turbomass, PerkinElmer, Inc., Waltham, MA, USA). The temperature program of the instrument was initially set at 40 °C for two minutes, followed by an increase at a rate of 5 °C per minute up to 300 °C. Compounds were identified by comparing spectra with the Adam Library [19] and the Wiley GC/MS Library [20]. In addition, the main compounds were drawn using ChemDraw Ultra 12.0.2 software (Perkinelmer, Waltham, MA, USA).

Statistical Analysis

Data from at least three separate experiments were used to calculate means and standard deviation (SD). The statistical analysis was performed using Analysis of Variance (ANOVA) in the GraphPad Prism 8.4.3 software (GraphPad Software, Inc., San Diego, CA, USA), with the Tukey post hoc test applied at $p < 0.05$.

Results

Cytotoxic Effects of H. Bacciferum Methanolic Extract and Fractions

In our investigation of the antiproliferative effects of *H. bacciferum*, we assessed the IC_{50} values for various extract fractions. Notable, the hexane extract emerged as the most promising, inhibiting the growth of cancer cells with IC_{50} values of 83.84 ± 0.48 µg/mL and 104.14 ± 1.2 µg/mL for A549 and MCF-7 respectively (Table 1). Moreover, our dose-dependent analysis of the impacts of hexane fraction on cell viability revealed a clear trend of cell growth inhibition, particularly in A549 lung cancer cells (Fig. 1). Given these results, hexane extract was selected for subsequent investigations.

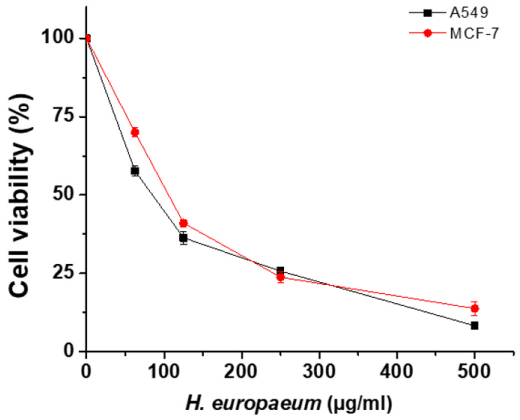

Fig. 1. Dose-response results of antiproliferative activities of hexane fraction using MTT assay. MTT, 3-(4,5-dimethylthiazol-2yl)-2,5 diphenyl tetrazolium bromide.

Cell Cycle Analysis

In this study, we conducted a comprehensive cell cycle analysis to evaluate the impact of *H. bacciferum* hexane extract on A549 cancer cells. Our investigation compared the treatment of the hexane extract to a control group (Fig. 2). Our results revealed the *H. bacciferum* hexane extract significantly increased the percentage of A549 cells in the G1 phase, with an increase of around 16% at the IC_{50} concentration and about 23.5% at the 2× IC_{50} concentration ($p < 0.0001$). Additionally, there was a significant decrease in the S phase from 24.1% to 15.7% for IC_{50}-treated cells and from 24.1% to 11.8% for 2× IC_{50}-treated cells, compared to the control. Moreover, in the G2/M phase, there was a significant decrease from 23.4% to 15.8% for IC_{50} treated cells ($p < 0.0001$) and from 23.4% to 12.4% for 2× IC_{50} treated cells ($p < 0.0001$), compared to the control.

H. Bacciferum Hexane Fraction Induces Apoptosis in A549 Cells

To understand the effects of *H. bacciferum* hexane extract on apoptosis, the A549 cells were treated with the extract, and the results were compared with a control group. The treatment of A549 cells with the *H. bacciferum* hexane fraction resulted in a significant increase in apoptotic cells (Fig. 3). Specifically, there was a substantial increase from 1.9% ± 0.1% to 38.3% ± 0.7% for the IC_{50} concentration ($p < 0.0001$), and this percentage further rose to 54.7% ± 1.13% for 2× IC_{50} treatment ($p < 0.0001$). The findings underscore the potent apoptotic effects of the *H. bacciferum* hexane fraction on A549 cells ($p < 0.0001$).

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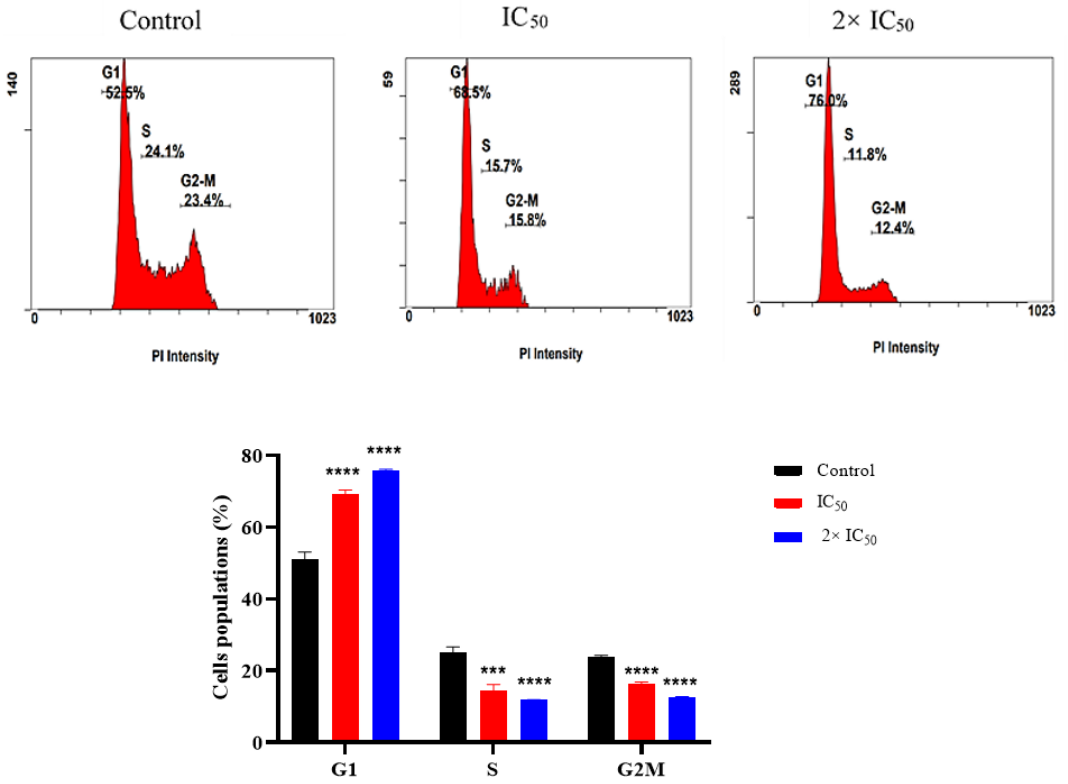

Fig. 2. Flow cytometry results of the *H. bacciferum* hexane fraction on the dispersion of the cell cycle in A549 cells. 83.84 (IC_{50}) and 167.68 ($2\times$ IC_{50}) µg/mL of the *H. bacciferum* hexane fraction were applied to A549 cells for 24 hours. ***$p < 0.001$ and ****$p < 0.0001$.

Composition of H. Bacciferum Hexane Fraction

The active compounds in *H. bacciferum* hexane fraction were assessed using chromatographic methods. The chromatogram is shown in Fig. 4. To the best of our knowledge, this is the first report on the phytochemical composition of *H. bacciferum* hexane fraction using gas chromatography/mass spectrometry (GC/MS).

Our analysis revealed the presence of seventeen compounds, collectively constituting 99.99% of the *H. bacciferum* hexane fraction. Notably, the primary constituents include methyl lineoleate (36.5%), linoleic acid (19.1%), and 3′-acetyllycopsamine (10.7%) (Table 2, Fig. 5). The remaining compounds accounted for approximately 33.69% of the total content, while the three primary compounds comprised approximately 66.3%. These findings provide a comprehensive overview of the composition of the *H. bacciferum* hexane fraction and offer valuable insights into its phytochemical profile.

Discussion

Cancer poses a global challenge, with increasing incidence rates and cancer-related deaths. As a result, immediate interventions are essential to eliminate or at least lessen the problem. Numerous studies have extensively investigated medicinal plants and their bioactive compounds for their potential in cancer treatment, highlighting their safety and effectiveness [21–25]. Notably, using plant-derived drugs offers the advantage of being non-toxic to normal cells. These drugs typically target cancer cells through various mechanisms, including DNA damage prevention, antioxidation, methylation regulation, disruption of mitosis, and inhibition of histone deacetylases (HDAC) [26]. This study was conducted in light of the cytotoxic potential of *H. bacciferum* extract and fractions, as well as the mechanisms behind cell cycle arrest and apoptosis induction. Increasing concentrations of the *H. bacciferum* methanolic extract and fractions were applied to A549 and MCF-7 cells. In a study to evaluate the cytotoxicity of *H. bacciferum* extracts on cancer cells, Canga *et al*. [27] observed that the chloroform extract inhibits the proliferation of HCT116 and DLD1 cells (IC_{50} = 95 µg/mL and 62 µg/mL, respectively), whereas the methanol extract had no effect on these cells [13]. These results differ somewhat from ours, as the chloroform extract had no effect on the cells used. However, the results values from their study were close to the results obtained in this study for the hexane extract. It is worth noting that the results can vary depending on the cancer cell types, extraction methods, and environmental factors [28,29].

The effect of the extract on cell cycle arrest or the cell cycle phase influenced by the extract was investigated to better understand the mechanical effect of hexane extract

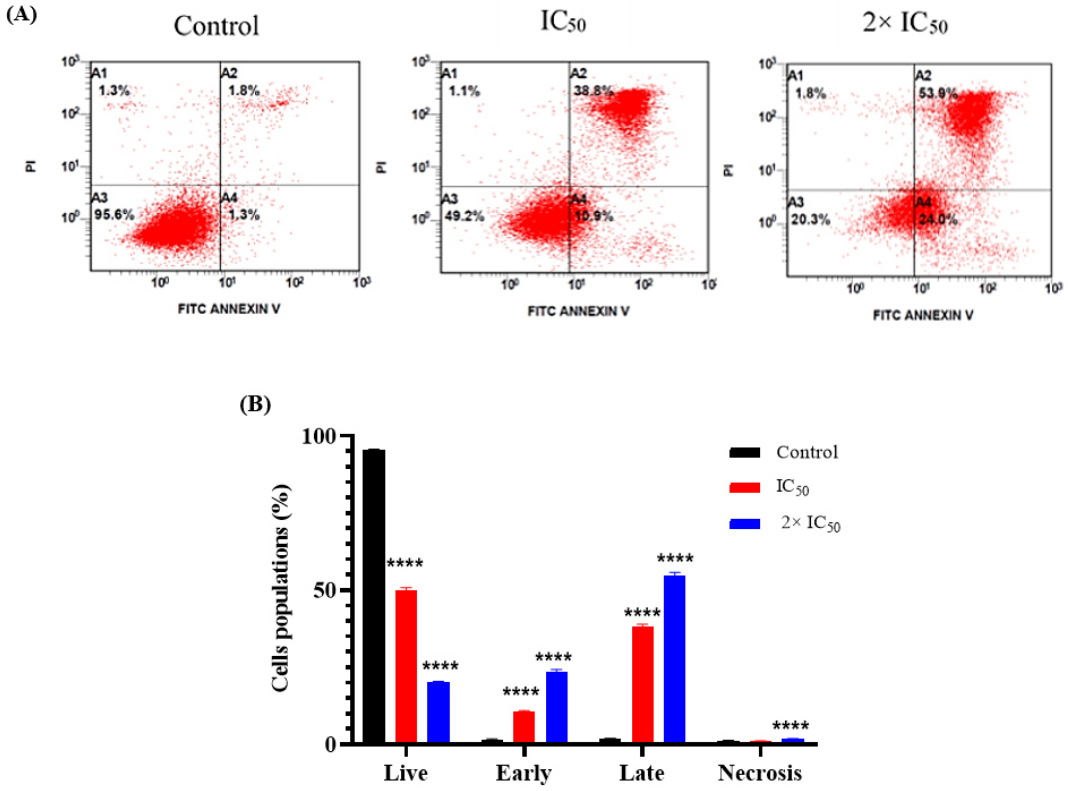

Fig. 3. Apoptosis assay of treated A549 cells using Annexin-V/PI. (A) Distribution of A549 cells without the treatment (control) and following treatment with IC_{50} and $2\times$ IC_{50} concentrations of *H. bacciferum* hexane fraction. (B) A bar graph showing cell distribution in control and treated A549 cells was analyzed using flow cytometry. ***$p < 0.001$ and ****$p < 0.0001$.

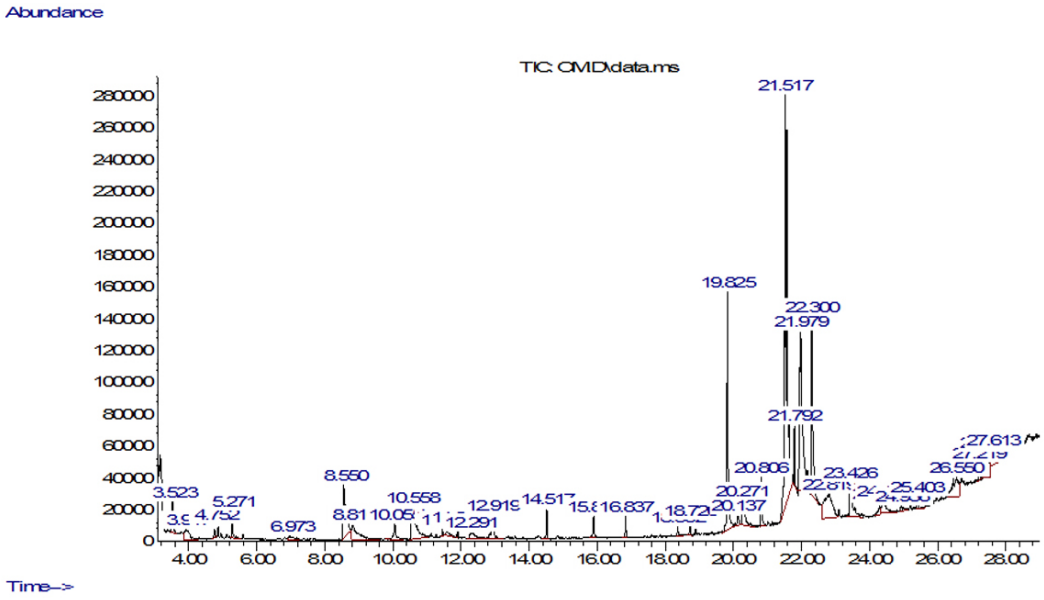

Fig. 4. Gas chromatography/mass spectrometry (GC/MS) chromatogram of *H. bacciferum* hexane extract.

on the inhibition of A549 cell growth. Our results demonstrate that the hexane extract of *H. bacciferum* inhibited the development of cancer cells by causing cell cycle arrest in the G1 phase, in a dose-dependent manner. This arresting action can be attributed to the active compounds in *H. bacciferum* hexane extract, such as methyl lineoleate, palmitic acid, and linoleic acid, which have been reported to disrupt or inhibit cell division [30–32].

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Table 2. GC/MS analysis and compound identification.

Compound name	Chemical formula	Molecular weight (g/mol)	RT (min)	%Area
4-O-Methyl-d-arabinose	$C_6H_{12}O_5$	164.16	3.917	2.08491517
5-Methylhexan-3-one	$C_7H_{14}O$	114.19	4.752	0.48785935
Indole	C_8H_7N	117.15	8.55	5.85678676
Nonylcyclopropane	$C_{12}H_{24}$	168.32	10.055	1.40059632
1-Ethoxy-2-methylpropane	$C_6H_{14}O$	102.17	10.558	5.59357327
1-Methyl-2-octylcyclopropane	$C_{12}H_{24}$	168.32	12.919	2.36611481
5-Octadecene, (E)-	$C_{18}H_{36}$	252.5	15.892	1.04787456
2-Tridecanol	$C_{13}H_{28}O$	200.36	18.362	0.46480327
Palmitic acid	$C_{16}H_{32}O_2$	256.42	20.271	3.0376985
Methyl lineoleate	$C_{19}H_{34}O_2$	294.5	21.517	36.5089961
Methyl isostearate	$C_{19}H_{38}O_2$	298.5	21.792	1.6974584
Linoleic acid	$C_{18}H_{32}O_2$	280.4	21.979	19.0997869
3′-Acetyllycopsamine	$C_{17}H_{27}NO_6$	341.4	22.3	10.7489604
Echinatine	$C_{15}H_{25}NO_5$	299.36	23.426	4.0096751
2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280.5	24.287	0.68664256
Methoxymethyl(triethyl)stannane	$C_8H_{20}OSn$	250.95	25.403	0.55645419
2(1H)-Naphthalenone, 4a,5,6,7,8,8a-hexahydro -7.alpha.-isopropyl-4a.beta.,8a.beta.-dimethyl-	$C_{14}H_{24}O$	208.34	26.55	4.35180435

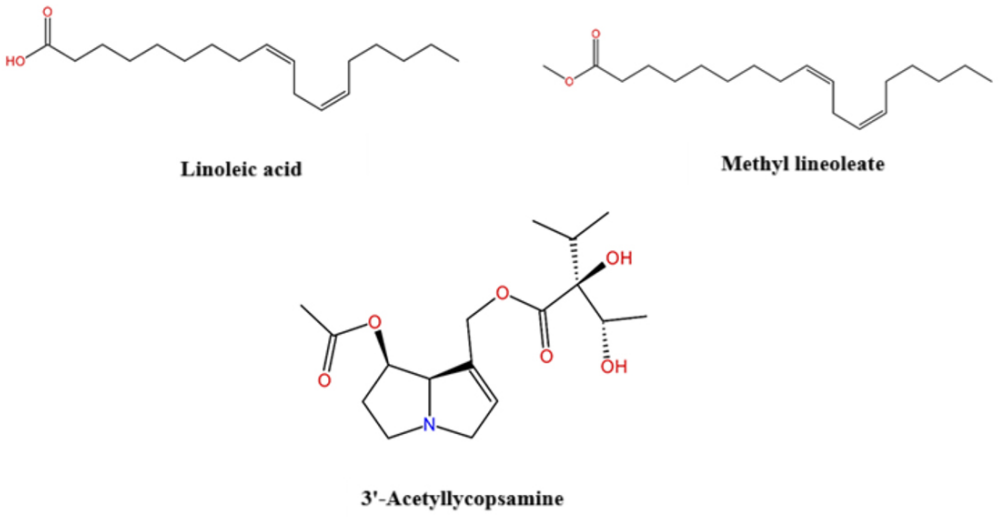

Fig. 5. Major compound identified in *H. bacciferum* hexane fraction.

The regulation of programmed cell death, from intrinsic or extrinsic pathways, is crucial for maintaining a balance between cell division and apoptosis. These pathways involve changes in plasma membrane permeability and the modulation of gene expression, including anti-apoptotic (*Bcl2*) and pro-apoptotic (*Bax*) genes, and activation of various caspases [33–35]. Recent research has placed great emphasis on finding new anticancer agents that can efficiently and specifically induce apoptosis in cancer cells [36]. In our study, we used the Annexin V-FITC/PI assay and flow cytometry analysis to examine whether the observed cell inhibition in A549 cells following treatment with the *H. bacciferum* hexane fraction was associated with apoptosis induction. The results demonstrated that the *H. bacciferum* hexane fraction induced programmed cell death (apoptosis) in a dose-dependent manner, affecting both the early and late stages. Importantly, our previous cell cycle assay revealed that cells treated with *H. bacciferum* hexane fraction accumulated in the G1 phase, suggesting that A549 cells undergo apoptosis via a p53-independent mechanism [37].

The phytochemical analysis results identified methyl lineoleate, linoleic acid and 3′-acetyllycopsamine as the main compounds in the *H. bacciferum* hexane fraction. The significance of these chemical compounds in treating various diseases, including cancer, lies in their antioxidant

properties, which counteract free radicals [38]. These compounds regulate molecular pathways implicated in cancer development and progression by enhancing antioxidant levels, inhibiting proliferation, preventing carcinogenesis, inducing cell cycle arrest, and promoting apoptosis while also regulating the immune system [39]. Numerous studies have demonstrated the impact of these compounds on cancer cell growth, apoptosis induction, and cell cycle arrest. For instance, research by Zhang *et al*. [40] revealed that linoleic and palmitic acids induced apoptosis in H4IIE cells. Palmitic acid, in particular, affects cell cycle progression in various ways, including reduced DNA synthesis, G2/M arrest, and failure of cytokinesis in N2a cells [32]. Moreover, these compounds induce apoptosis in multiple cancer cell lines by activating signaling pathways such as MAPK, AMPK/Akt/mTOR, miR-129-3p/Smad3, and estrogen receptor alpha [41–43].

Conclusions

Our study revealed that the *H. bacciferum* hexane fraction is rich in three primary chemicals, which are methyl lineoleate, linoleic acid, and 3′-acetyllycopsamine. This is the first work through which the phytochemical components and anti-cancer mechanisms of *H. bacciferum* are explained. Moreover, this fraction can halt the cell cycle and induce apoptosis in A549 and MCF-7 cancer cells, effectively impeding their proliferative abilities. These observations suggest that this fraction may trigger G1 arrest and apoptosis in A549 cells through a p53-independent mechanism. Consequently, by inhibiting the cell cycle in the G1 phase, the *H. bacciferum* hexane fraction holds promise as a potential therapeutic agent for malignant disorders.

Availability of Data and Materials

The data used to support the findings of this study are available from the corresponding author upon request.

Author Contributions

OAk: Conceptualization, methodology, formal analysis, data curation and writing—original draft preparation. ASA: methodology, formal analysis, review and editing. ON: formal analysis, review and editing. HI: methodology, formal analysis, data curation and writing—original draft preparation. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Imtara H, Kmail A, Touzani S, Khader M, Hamarshi H, Saad B, *et al*. Chemical Analysis and Cytotoxic and Cytostatic Effects of Twelve Honey Samples Collected from Different Regions in Morocco and Palestine. Evidence-Based Complementary and Alternative Medicine. 2019; 2019: 8768210.

[2] Ritchie H, Spooner F, Roser M. Causes of Death. Our World in Data. 2018.

[3] World Health Organization. Cancer. 2022. Available at: https://www.who.int/news-room/fact-sheets/detail/cancer (Accessed: 28 February 2023).

[4] Arruebo M, Vilaboa N, Sáez-Gutierrez B, Lambea J, Tres A, Valladares M, *et al*. Assessment of the evolution of cancer treatment therapies. Cancers. 2011; 3: 3279–3330.

[5] Debela DT, Muzazu SG, Heraro KD, Ndalama MT, Mesele BW, Haile DC, *et al*. New approaches and procedures for cancer treatment: Current perspectives. SAGE Open Medicine. 2021; 9: 20503121211034366.

[6] National Institute of Health. Side Effects of Cancer Treatment – NCI. 2015. Available at: https://www.cancer.gov/about-cancer/treatment/side-effects (Accessed: 28 February 2023).

[7] Touzani S, Embaslat W, Imtara H, Kmail A, Kadan S, Zaid H, *et al*. *In Vitro* Evaluation of the Potential Use of Propolis as a Multitarget Therapeutic Product: Physicochemical Properties, Chemical Composition, and Immunomodulatory, Antibacterial, and Anticancer Properties. BioMed Research International. 2019; 2019: 4836378.

[8] El Omari N, Bakha M, Imtara H, Guaouguaoua FE, Balahbib A, Zengin G, *et al*. Anticancer mechanisms of phytochemical compounds: focusing on epigenetic targets. Environmental Science and Pollution Research International. 2021; 28: 47869–47903.

[9] Kopustinskiene DM, Jakstas V, Savickas A, Bernatoniene J. Flavonoids as Anticancer Agents. Nutrients. 2020; 12: 457.

[10] Bhosale PB, Ha SE, Vetrivel P, Kim HH, Kim SM, Kim GS. Functions of polyphenols and its anticancer properties in biomedical research: a narrative review. Translational Cancer Research. 2020; 9: 7619–7631.

[11] Spatafora C, Tringali C. Natural-derived polyphenols as potential anticancer agents. Anti-cancer Agents in Medicinal Chemistry. 2012; 12: 902–918.

[12] Ahmad S, AbdEl-Salam NM, Ullah R. In Vitro Antimicrobial Bioassays, DPPH Radical Scavenging Activity, and FTIR Spectroscopy Analysis of Heliotropium bacciferum. BioMed Research International. 2016; 2016: 3818945.

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

[13] Aïssaoui H, Mencherini T, Esposito T, De Tommasi N, Gazzerro P, Benayache S, *et al*. *Heliotropium bacciferum* Forssk. (Boraginaceae) extracts: chemical constituents, antioxidant activity and cytotoxic effect in human cancer cell lines. Natural Product Research. 2019; 33: 1813–1818.

[14] Fakhry AM, Migahid MMA, Anazi HK. Herbal Remedies Used in the Treatment of Scorpion Stings in Saudi Arabia. Global Journal of Medicinal Plant Research. 2017; 5: 1–8.

[15] Ahmad S, Ahmad S, Bibi I, AbdEl-Salam N, Hussain H, Ishaq M, *et al*. Antibacterial and Antifungal Activities of the Extract and Fractions of Aerial Parts of Heliotropium Bacciferum. African Journal of Traditional, Complementary and Alternative Medicines. 2015; 12: 32–35.

[16] Fathalipour-Rayeni H, Forootanfar H, Khazaeli P, Mehrabani M, Rahimi HR, Shakibaie M, *et al*. Evaluation of antioxidant potential of Heliotropium bacciferum Forssk extract and wound healing activity of its topical formulation in rat. Annales Pharmaceutiques Francaises. 2022; 80: 280–290.

[17] Nasr FA, Shahat AA, Alqahtani AS, Ahmed MZ, Qamar W, Al-Mishari AA, *et al*. Centaurea bruguierana inhibits cell proliferation, causes cell cycle arrest, and induces apoptosis in human MCF-7 breast carcinoma cells. Molecular Biology Reports. 2020; 47: 6043–6051.

[18] S Alqahtani A, Nasr FA, Noman OM, Farooq M, Alhawassi T, Qamar W, *et al*. Cytotoxic Evaluation and Anti-Angiogenic Effects of Two Furano-Sesquiterpenoids from *Commiphora myrrh* Resin. Molecules. 2020; 25: 1318.

[19] Sparkman OD. Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy Robert P. Adams. Journal of the American Society for Mass Spectrometry. 2005; 16: 1902–1903.

[20] Wiley. Wiley/NBS Registry of Mass Spectral Data. 2023. Available at: https://analyticalscience.wiley.com/do/10.1002/sepspec.9780471628866/ (Accessed: 2 November 2023).

[21] George BP, Chandran R, Abrahamse H. Role of Phytochemicals in Cancer Chemoprevention: Insights. Antioxidants. 2021; 10: 1455.

[22] Regassa H, Sourirajan A, Kumar V, Pandey S, Kumar D, Dev K. A Review of Medicinal Plants of the Himalayas with Anti-Proliferative Activity for the Treatment of Various Cancers. Cancers. 2022; 14: 3898.

[23] Kuruppu AI, Paranagama P, Goonasekara CL. Medicinal plants commonly used against cancer in traditional medicine formulae in Sri Lanka. Saudi Pharmaceutical Journal. 2019; 27: 565–573.

[24] Choudhari AS, Mandave PC, Deshpande M, Ranjekar P, Prakash O. Phytochemicals in Cancer Treatment: From Preclinical Studies to Clinical Practice. Frontiers in Pharmacology. 2020; 10: 1614.

[25] Gaobotse G, Venkataraman S, Brown PD, Masisi K, Kwape TE, Nkwe DO, *et al*. The use of African medicinal plants in cancer management. Frontiers in Pharmacology. 2023; 14: 1122388.

[26] Greenwell M, Rahman PKSM. Medicinal Plants: Their Use in Anticancer Treatment. International Journal of Pharmaceutical Sciences and Research. 2015; 6: 4103–4112.

[27] Canga I, Vita P, Oliveira AI, Castro MÁ, Pinho C. In Vitro Cytotoxic Activity of African Plants: A Review. Molecules. 2022; 27: 4989.

[28] Bennour N, Mighri H, Eljani H, Zammouri T, Akrout A. Effect of Solvent Evaporation Method on Phenolic Compounds and the Antioxidant Activity of Moringa Oleifera Cultivated in Southern Tunisia. South African Journal of Botany. 2020; 129: 181–190.

[29] Uğur D, Güneş H, Güneş F, Mammadov R. Cytotoxic Activities of Certain Medicinal Plants on Different Cancer Cell Lines. Turkish Journal of Pharmaceutical Sciences. 2017; 14: 222–230.

[30] Kemp MQ, Jeffy BD, Romagnolo DF. Conjugated linoleic acid inhibits cell proliferation through a p53-dependent mechanism: effects on the expression of G1-restriction points in breast and colon cancer cells. The Journal of Nutrition. 2003; 133: 3670–3677.

[31] FRANZ J, COLE BT. Effects of ultraviolet-irradiated methyl linolenate on cell division and respiration in Saccharomyces cerevisiae. Archives of Biochemistry and Biophysics. 1962; 96: 382–385.

[32] Urso CJ, Zhou H. Palmitic acid-induced defects in cell cycle progression and cytokinesis in Neuro-2a cells. Cell Cycle. 2022; 21: 1048–1057.

[33] Lucken-Ardjomande S, Martinou JC. Regulation of Bcl-2 proteins and of the permeability of the outer mitochondrial membrane. Comptes Rendus Biologies. 2005; 328: 616–631.

[34] Shamas-Din A, Kale J, Leber B, Andrews DW. Mechanisms of action of Bcl-2 family proteins. Cold Spring Harbor Perspectives in Biology. 2013; 5: a008714.

[35] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144: 646–674.

[36] Kaur V, Kumar M, Kumar A, Kaur K, Dhillon VS, Kaur S. Pharmacotherapeutic potential of phytochemicals: Implications in cancer chemoprevention and future perspectives. Biomedicine & Pharmacotherapy. 2018; 97: 564–586.

[37] Park HY, Park SH, Jeong JW, Yoon D, Han MH, Lee DS, *et al*. Induction of p53-Independent Apoptosis and G1 Cell Cycle Arrest by Fucoidan in HCT116 Human Colorectal Carcinoma Cells. Marine Drugs. 2017; 15: 154.

[38] Alzandi AA, Taher EA, Al-Sagheer NA, Al-Khulaidi AW, Azizi M, Naguib DM. Phytochemical Components, Antioxidant and Anticancer Activity of 18 Major Medicinal Plants in Albaha Region, Saudi Arabia. Biocatalysis and Agricultural Biotechnology. 2021; 34: 102020.

[39] Kmail A, Lyoussi B, Zaid H , Imtara H , Saad B. In vitro evaluation of anti-inflammatory and antioxidant effects of Asparagus aphyllus L., Crataegus azarolus L., and Ephedra alata Decne.in monocultures and co-cultures of HepG2 and THP-1-derived macrophages. Pharmacognosy Communications. 2017: 7: 24-33.

[40] Zhang Y, Xue R, Zhang Z, Yang X, Shi H. Palmitic and linoleic acids induce ER stress and apoptosis in hepatoma cells. Lipids in Health and Disease. 2012; 11: 1.

[41] Mahajna S, Kadan S, Tietel Z, Saad B, Khasib S, Tumeh A, *et al*. In Vitro Evaluation of Chemically Analyzed *Hypericum Triquetrifolium* Extract Efficacy in Apoptosis Induction and Cell Cycle Arrest of the HCT-116 Colon Cancer Cell Line. Molecules. 2019; 24: 4139.

[42] Zou Y, Kong M. Tetrahydroxy stilbene glucoside alleviates palmitic acid-induced inflammation and apoptosis in cardiomyocytes by regulating miR-129-3p/Smad3 signaling. Cellular & Molecular Biology Letters. 2019; 24: 5.

[43] Liang Z, Yuan Z, Guo J, Wu J, Yi J, Deng J, *et al*. *Ganoderma lucidum* Polysaccharides Prevent Palmitic Acid-Evoked Apoptosis and Autophagy in Intestinal Porcine Epithelial Cell Line via Restoration of Mitochondrial Function and Regulation of MAPK and AMPK/Akt/mTOR Signaling Pathway. International Journal of Molecular Sciences. 2019; 20: 478.