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Research Article

Investigating the Potential Cytotoxic Effects and Phytochemical Profile of *Ruta chalepensis* Aqueous, Acetone, and Hexane Extracts

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Cancer remains a leading cause of mortality worldwide, driving the urgent need for safer and more effective treatment options. Herbal medicine, with its diverse bioactive compounds, offers a promising source of novel anticancer agents that may enhance therapeutic efficacy while minimizing adverse effects. This study explores the phytochemical composition and cytotoxic potential of *Ruta chalepensis* extracts (acetone, aqueous, and hexane) in an in vitro model. According to high-performance liquid chromatography (HPLC), the acetone and aqueous extracts were high in both flavonoids and monoterpenes, whereas the hexane extract was high in fatty acids and triterpenoids. The cytotoxic effects of these extracts were tested using MTT assay against human lung (A549) and prostate (PC3) cancer cell lines, as well as normal fibroblasts (CCD-1064SK). With IC_{50} values of 43.78 ± 1.18 μg/mL for A549 and 65.96 ± 2.33 μg/mL for PC3, the acetone extract showed the most potent anticancer activity among the examined extracts. These findings suggest that *R. chalepensis* acetone extract has the potential to be an effective anticancer agent, which requires additional research into its bioactive components and mechanisms of action.

Keywords: A549; cell viability IC_{50}; cytotoxicity; MTT assay; PC3; *Ruta chalepensis*

1. Introduction

Cancer represents a heterogeneous group of disorders characterized by abnormal and uncontrolled cellular proliferation, alteration of regulatory pathways, and the ability to invade surrounding tissues and metastasize to distant organs [1]. It remains the major cause of morbidity and mortality worldwide. Approximately two-thirds of all new cases and fatalities globally in 2022 were caused by 10 different types of cancer, according to recent estimates by the Global Cancer Observatory [2]. Globally, lung cancer was the most frequently diagnosed cancer, responsible for 2.5 million new cases, accounting for 12.4% of all newly reported cancers. It was followed by female breast cancer with 2.3 million cases (11.6%), colorectal cancer with 1.9 million cases (9.6%), prostate cancer with 1.5 million cases (7.3%), and stomach cancer with 970,000 cases (4.9%) [2]. These statistics highlight the critical need for constant development of therapeutic medicines that provide higher efficacy while minimizing adverse effects [3]. Despite developments in traditional cancer treatments including radiation, chemotherapy, and targeted therapies, limitations such as multidrug resistance, nonspecific toxicity, and lack of selectivity continue to impede their overall success [4, 5]. As a result, there is an increased interest in complementary and alternative therapies, particularly those derived from natural sources [6]. In recent years, medicinal plants have received increased attention as possible sources of bioactive compounds with potent anticancer properties. Many of these compounds have been shown to affect critical molecular pathways involved in tumor genesis, progression, and metastasis, offering

a promising avenue for the discovery and development of safer, more specific anticancer treatments [6, 7].

Medicinal plants have emerged as promising candidates in cancer research due to their rich repertoire of bioactive compounds [8, 9]. Several plant-derived compounds, such as paclitaxel from *Taxus brevifolia* and vinblastine from *Catharanthus roseus*, have been successfully integrated into clinical cancer treatments, demonstrating the potential of phytochemicals in drug discovery [10]. Many medicinal plants' full therapeutic potential and safety profiles remain underexplored, particularly in cytotoxicity and possible adverse effects [11].

Ruta chalepensis is a perennial aromatic herbaceous plant belonging to the Rutaceae family. Its many pharmacological qualities have historically led to its use in folk medicine, including anti-inflammatory, antispasmodic, antimicrobial, and analgesic effects [12]. The plant's therapeutic potential is attributed to its complex phytochemical profile, encompassing alkaloids, flavonoids, coumarins, phenolics, and essential oils [13].

In previous research, *R. chalepensis* exerted many biological activities like antioxidant [12, 14, 15], cytotoxic activity [15, 16], antimicrobial properties [17, 18], and antihemolytic [15]. The choice of extraction solvents significantly impacts the bioactivity of plant extracts, as different solvents extract distinct classes of phytochemicals [19]. Thus, this work intends to study the anticancer effect of *R. chalepensis*.

For the first time, this study aims to examine the anticancer effects of aqueous, acetone, and hexane extracts of *Ruta chalepensis* on lung (A549) and prostate (PC3) cancer cell lines in contrast to noncancerous fibroblast cells. Phytochemical profiling will be performed with high-performance liquid chromatography (HPLC), and cytotoxicity will be assessed using 3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) tests. The findings aim to highlight *R. chalepensis'* therapeutic potential and promote its usage in plant-based anticancer treatments.

2. Materials and Methods

2.1. Collection of Plants. Aerial samples of *Ruta chalepensis* were effectively gathered from Amman County in Jordan in May 2024. Prof. Sawsan Oran thoroughly identified and authenticated these materials. Then a voucher specimen (No. RC/2024/2) was kept in the Herbarium of the Department of Biological Sciences at the University of Jordan, in Amman, Jordan.

2.2. Preparation of Extracts. Initially, the plant's leaves, stems, and flowers were air-dried until desiccated. Once thoroughly dried, the plant material was finely pulverized to enhance the extraction efficiency. Next, a solvent—either distilled water, acetone, or hexane—was selected to immerse 10 g of the powdered plant material in 100 mL of the chosen solvent for 3 days. After immersion, the mixture passed through the Whatman filter paper (number one). Next, the resultant liquid extract was run through a rotary evaporator, where the solvent evaporated under 50°C under supervision. At last, the filtered extracts were left to completely dry; the dried extract was kept at 4°C for subsequent use and research [20].

2.3. HPLC Analysis. The phytochemical analysis of the examined extracts was performed using HPLC coupled with mass spectrometry (MS) (Shimadzu, Japan). This setup included the Control Bus Module-20A, Column Oven-30A, Liquid Chromatograph-30AD, Auto Sampler SIL-30AC, and LCMS-8030 Triple Quadrupole Mass Spectrometer.

25 mg of the plant extract was dissolved in 1 mL of methanol, and 10 μL of this solution was injected into the HPLC system. The chromatographic separation was carried out using ACE 5 C18 column (250 × 4.6 mm, 5 μm), preserving a temperature of 30°C and a flow rate of 0.8 mL/min. A gradient elution technique was run using a mobile phase including water with 0.1% acetic acid (A), methanol with 0.1% acetic acid (B), and acetonitrile with 0.1% acetic acid (C). The mobile phase's starting composition was 80:12:8; however, over 40–45 min, it was progressively changed to 25:60:15. After reaching this final composition, the system was reverted to the initial ratio (80:12:8) for an additional 5 min to facilitate column reconditioning.

Prior to the HPLC injections, both the extract samples and the solvents used for the mobile phase were filtered through a 0.22 μm filter system (Millipore Corp., Billerica, MA, USA) to ensure purity. Each sample was analyzed in triplicate.

2.4. Cell Culture. Prostate cancer PC3, human lung cancer A549, and normal skin fibroblast CCD-1064SK were acquired from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultivated by ATCC protocols. While fibroblasts were cultivated in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma, USA), A549 and PC3 cell lines were cultivated in RPMI1640 (Sigma, USA). The cells were cultured in T25 culture flasks with the proper media supplemented with 10% fetal bovine serum (Cytiva, Austria), 1% penicillin-streptomycin, 1% L-glutamine, and 1% 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), all of which were acquired from EuroClone S.P.A., Italy. The cells were kept in a humidified environment with 5% CO2 at 37°C. Every 2 days, these cells are continuously inspected and validated.

2.5. Cell Viability Assay. Cell viability was assessed via the MTT assay (ATCC® 30–1010 K). Cells were seeded in 96-well plates (SPL Life Sciences Co., Korea) at a density of 10,000 cells per well and incubated for 24 h. The cells were treated with different extract concentrations (3.125–200 μg/mL). Doxorubicin at doses 0.1–10 μg/mL was used as a reference drug. All samples were dissolved in DMSO, and the concentration did not exceed 0.1%. All samples were measured in five repetitions. Fifteen microliters of MTT reagent was added to each well and incubated for 4 hours at 37°C. After removing the culture media, 100 μL of stop solution was used to solubilize the formazan crystal

Table 1: Chemical composition of *R. chalepensis* aqueous *extract*.

No	Compound name	% composition	Retention time (min)
1	2,2-Dimethyl-3-methylenebicyclo[2.2.1]heptane	8.0	0.9
2	1-Methyl-4-(1-methylethenyl)cyclohexene	6.0	1.2
3	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-	5.3	2.1
4	Unknown	7.3	6.9
5	3,7-Dimethyl-1,6-octadien-3-ol	5.8	13.9
6	7-Hydroxycoumarin	10.0	15
7	3,4-Dihydroxycinnamic acid	8.7	15.9
8	7-Hydroxy-6-methoxycoumarin	9.7	19.3
9	4-Hydroxy-3-methoxycinnamic acid	6.0	20.0
10	Rutamarin	11.4	21.0
11	3,3′,4′,5,7-Pentahydroxyflavone	9.2	23.1
12	5-O-Caffeoylquinic acid	7.3	25.3
13	Rutin	5.3	27.0
	Total identified	92.7%	
	Phytochemical classification		
	Monoterpene	25.1%	
	Phenol	22.0%	
	Coumarin	19.7%	
	Flavonoid	14.5%	
	Alkaloid	11.4%	

[21]. The absorbance at 570 nm was quantified utilizing an HTX multimode microplate reader (BioTek, USA). The 50% inhibitory concentration (IC_{50}) was determined utilizing GraphPad Prism 8.0.2. The selectivity index (SI) was calculated by dividing the IC_{50} value for normal fibroblast cells (CCD-1064SK) by the IC_{50} value for the malignant cell lines (A549 and PC3). SI = IC_{50} of each sample on normal cells (CCD-1064SK) divided by IC_{50} of each extract on cancerous cell lines (A549 and PC3). The SI reflects the sample's selectivity toward the examined cell lines. Samples with SI ≥ 2 were considered highly selective for cancerous cells [22].

2.6. Statistical Analysis. Statistical analysis was accomplished using GraphPad Prism 8.0.2 using two-way ANOVA analysis. Results are presented as mean ± SEM, and the p value is expressed as $p^{*} < 0.05$, $p^{**} < 0.01$, $p^{***} < 0.001$, and $p^{****} < 0.0001$.

3. Results

3.1. Phytochemical Analysis. A comprehensive chemical analysis determined the composition of *R. chalepensis* extracts prepared with aqueous, acetone, and hexane solvents. Tables 1, 2, and 3 present the findings, indicating significant variations in component distribution based on solvent polarity. HPLC yielded retention times and peak intensities for each identified compound (Figures 1(a), 1(b), and 1(c)).

The aqueous extract demonstrated significant levels of monoterpenes (25.1%) and phenols (22%), with coumarins (19.7%) and flavonoids (14.5%) present in lesser amounts. Rutamarin (11.4%) was the predominant compound in the alkaloid fraction, followed by 7-hydroxycoumarin (10%) and 3,3′,4′,5,7-pentahydroxyflavone (9.2%), which were also significant constituents. A total of 92.7% of the extract's composition was comprised of identified compounds (Table 1).

In the acetone extract, monoterpenes dominated at 35.7%, with flavonoids (24%) and alkaloids (13.5%) also present in significant proportions. The primary compounds identified were 4-thujene (8.9%), sophoretin (8%), and flavonol, in addition to furanocoumarins (5.3%) and phenolic acids (4.6%). 88.1% accounted for the identified phytochemicals of the extract (Table 2).

High quantities of fatty acids (20.4%) and phenylpropenes (14.9%), together with considerable amounts of triterpenoids (10.8%), and phytosterols (9%) were found in the hexane extract. The most plentiful chemical was oleanic acid (10.8%), then hexadecanoic acid (9.8%) and anise camphor (8%). Sesquiterpene showed a small amount of β-germacrene (3.4%) and β-caryophyllene (2%) (Table 3).

Generally, the solvent-specific chemical profiles of *R. chalepensis* extracts show that while the hexane extract is concentrated in fatty acids and triterpenoids, aqueous and acetone extracts are mostly constituted of monoterpenes and flavonoids. These differences highlight how phytochemical extraction and possible biological consequences depend on solvent polarity.

3.2. The Cytotoxic Activity of Various R. chalepensis Extracts. The results revealed that *R. chalepensis* extracts exhibited cytotoxic effects against A549 and PC3 cancer cell lines (Table 4 and Figure 2). The acetone extract showed the most pronounced activity (IC_{50}: 43.78 ± 1.18 μg/mL for A549, 65.96 ± 2.33 μg/mL for PC3) and greater selectivity compared to other extracts. The aqueous and hexane extracts exhibited moderate cytotoxicity but with reduced selectivity. Doxorubicin exhibited the greatest potency (IC_{50}: 0.93 ± 0.094 μg/mL for A549, 2.71 ± 0.70 μg/mL for PC3) although showing much greater toxicity to normal fibroblasts. The results indicate that the acetone extract of *R. chalepensis* may possess significant anticancer potential with less toxicity to normal cells.

Table 2: Chemical composition of *R. chalepensis* acetone extract.

No	Compound name	% composition	Retention time (min)
1	D-Limonene	3.0	1.2
2	Pinene	4.9	2.8
3	4-Thujene	8.9	4.1
4	Methyl chavicol	3.0	5.0
5	Anise camphor	2.0	5.1
6	2-Isopropyl-5-methylphenol	4.5	6.1
7	2-Camphanone	5.7	6.4
8	3-Carvomenthenone	2.1	9.1
9	Borneo camphor	1.6	9.4
10	Isobornyl alcohol	3.0	11.1
11	Unknown	6.1	13.9
12	Hexahydrothymol	2.0	15.0
13	3,4,5-Trihydroxybenzoic acid	3.2	15.4
14	3,4-Dihydroxycinnamic acid	1.4	16.1
15	Unknown	2.3	16.9
16	5-Methoxypsoralen	5.3	19.9
17	Fagarine	6.2	21.1
18	Haplopine	7.3	22.1
19	Sophoretin	8.0	22.8
20	Cannabiscetin	6.5	25.2
21	Rutamarin	5.6	25.5
22	Unknown	3.5	26.1
23	Sophorin	3.9	27.1
Total identified		88.1%	
Phytochemical classification			
Monoterpene		35.7%	
Flavonoid		24.0%	
Alkaloid		13.5%	
Furanocoumarin		5.3%	
Phenylpropene		5.0%	
Phenol		4.6%	

Table 3: Chemical composition of *R. chalepensis* hexane extract.

No	Compound name	% composition	Retention time (min)
1	(R)-Limonene	4.5	5.3
2	Sabinene	6.2	4.8
3	Methyl chavicol	6.9	21.3
4	Anise camphor	8.0	18.1
5	3-Carvomenthenone	9.2	9.2
6	β-Germacrene	3.4	26.1
7	β-Caryophyllene	2.0	26.3
8	5-Methoxypsoralen	4.5	3.9
9	Hexadecanoic acid	9.8	25
10	cis-9,12-Octadecadienoic acid	5.3	23.8
11	Unknown	4.0	17.3
12	Octadecanoic acid	5.3	25.4
13	Rutamarin	6.6	1.2
14	Stigmasterin	3.7	23.4
15	β-Sitosterol	5.3	22.4
16	Oleanic acid	10.8	27.9
17	Unknown	4.5	28.4
Total identified		91.5%	
Phytochemical classification			
Fatty acid		20.4%	
Monoterpene		19.9%	
Phenylpropene		14.9%	
Triterpenoid		10.8%	
Phytosterol		9.0%	
Flavonoid		6.6%	
Sesquiterpene		5.4%	
Furanocoumarin		4.5%	

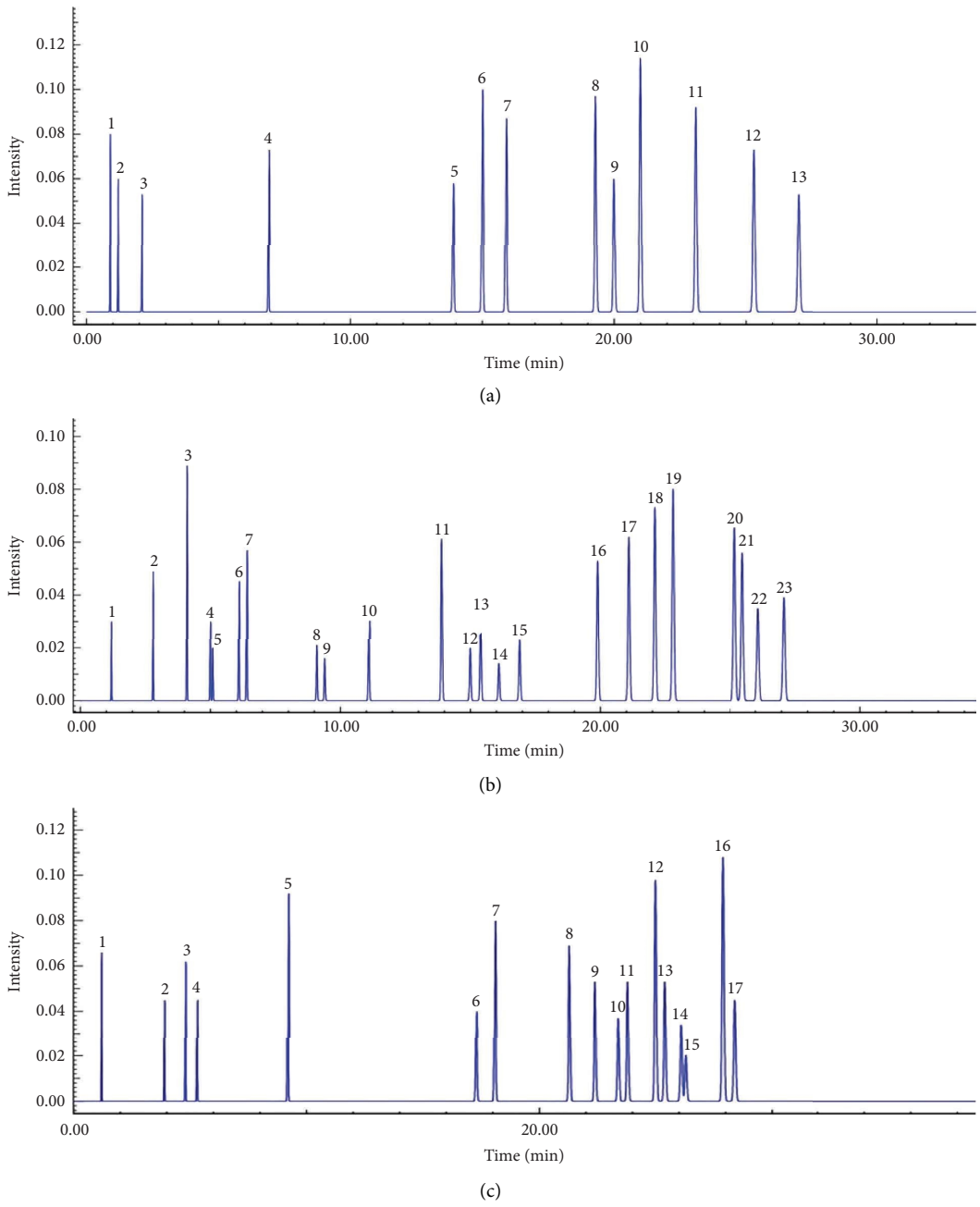


Figure 1: HPLC chromatogram of *R. chalepensis* (a) aqueous extract, (b) acetone extract, and (c) hexane extract.

4. Discussion

Synthetic drugs used to treat cancer are often the cause of major harmful effects. Medicinal plants offer reasonably priced, less adverse effects than synthetic drugs, readily available sources of phytochemicals with potent anticancer action as a good replacement. Among the substances that have shown promise in stopping metastases, cancer cell proliferation, and causing death include flavonoids, alkaloids, terpenoids, phenols, and saponins [23].

The phytochemicals present in *R. chalepensis* extracts reveal the large amount of secondary metabolites as therapeutic agents for enhancing health and avoiding diseases. The chemical composition that raises a plant's medicinal potential influences climate, soil, genotype, developmental stage, and other biological and environmental factors [24]. Choosing the suitable extraction solvent will also help maximize these phytochemicals' concentration and efficacy [25], opening the path for creative plant-based medicinal approaches.

The chemical analysis in this study shows different bioactive profiles that may have anticancer effects. The aqueous extract, rich in monoterpenes, phenols, and coumarins, includes rutamarin, known for inducing apoptosis and

Table 4: IC_{50} values (μg/mL) (mean ± SEM) and selectivity index (SI) of *R. chalepensis* acetone, aqueous, and hexane extracts and doxorubicin against the human lung and prostatic cancer cell lines compared with normal fibroblast cells.

Compounds	IC_{50} lung cancer (A549)	SI	IC_{50} prostatic cancer (PC3)	SI	IC_{50} normal skin fibroblast (CCD-1064SK)
R. chalepensis acetone extract	43.78 ± 1.18^{c}	2.23	65.96 ± 2.33^{c}	1.48	97.47 ± 2.21
R. chalepensis aqueous extract	51.17 ± 0.87^{c}	1.80	71.52 ± 2.16^{c}	1.29	92.30 ± 1.24
R. chalepensis hexane extract	70.41 ± 0.90^{c}	1.83	72.64 ± 1.17^{c}	1.78	129.0 ± 2.03
Doxorubicin	0.93 ± 0.094^{a}	1.06	2.71 ± 0.70^{b}	0.37	0.99 ± 0.05

Note: Values are the mean of five replicates ± SEM. Statistical analysis was performed using two-way ANOVA followed by Dunnett's multiple comparisons test.
[a] $p^{**} < 0.01$.
[b] $p^{***} < 0.001$.
[c] $p^{****} < 0.0001$.

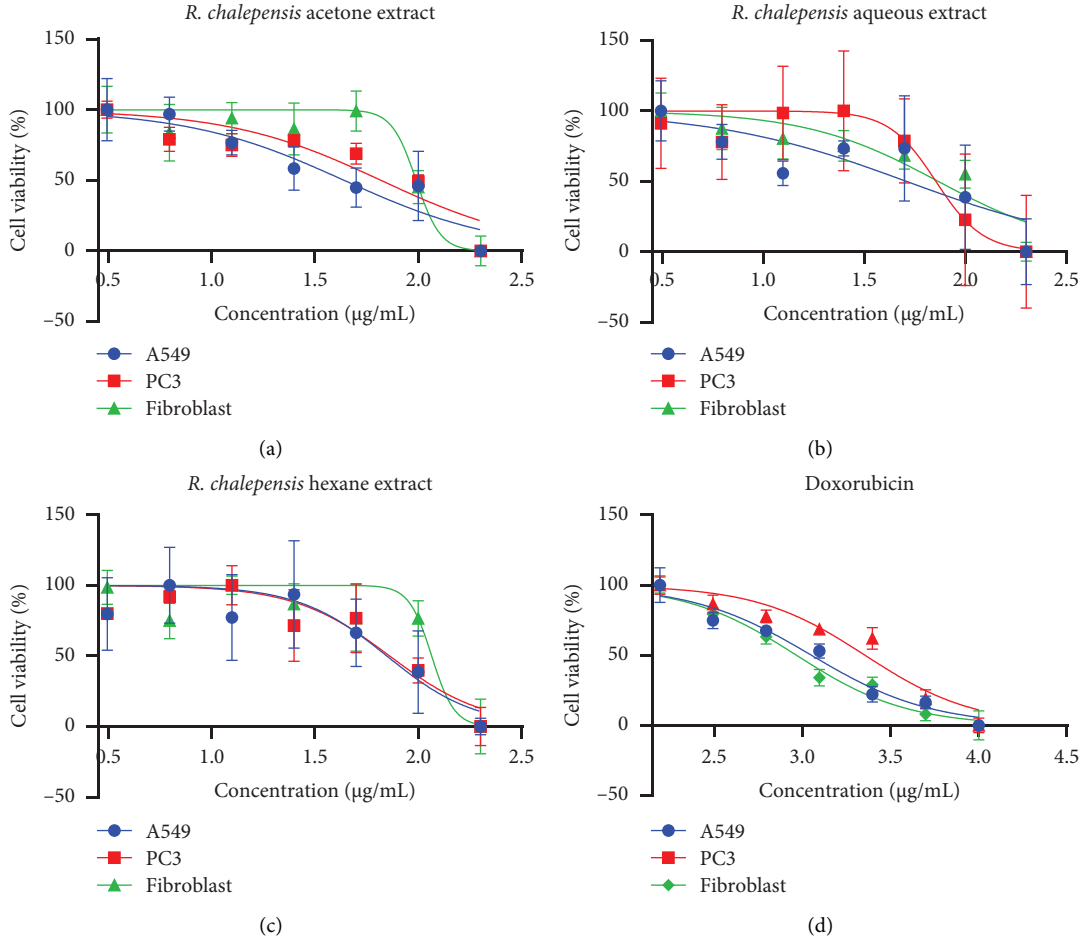


Figure 2: Comparative cytotoxic activity of (a) *R. chalepensis* acetone, (b) *R. chalepensis* aqueous, and (c) *R. chalepensis* hexane extracts and (d) doxorubicin against the human lung (A549), prostatic cancer (PC3), and normal skin fibroblast (CCD-1064SK) cell lines.

inhibiting cancer cell proliferation [26]. It also has 3,3′,4′,5,7-pentahydroxyflavone, which is known for its protective and cytotoxic properties [27]. 7-hydroxycoumarin also exists in it, which intercalates DNA and stops the cancer cell cycle [28].

The acetone extract is defined by the presence of monoterpenes, flavonoids, and alkaloids. For instance, 4-thujene (alkaloids) exhibited cytotoxic and antiproliferative characteristics, as described by Alexa et al. [29], whereas sophoretin is documented for its capacity to induce apoptosis and inhibit metastasis [30]. Furanocoumarins can inhibit cancer by DNA crosslinking [31].

The hexane extract is rich in fatty acids and triterpenoids. It comprises oleanic acid, which inhibits the migration of cancer cells and induces apoptosis [32], together with hexadecanoic acid, which prevents the proliferation of cancer cells [33]. These products demonstrate various applications in cancer treatment, emphasizing their distinct phytochemical compositions.

The findings indicate that *R. chalepensis* extracts exhibit notable cytotoxic effects against A549 and PC3 cancer cell lines, with the acetone extract demonstrating the highest activity and selectivity. The moderate cytotoxicity observed in the aqueous and hexane extracts, along with their lower SIs, suggests a weaker therapeutic potential compared to the acetone extract. In contrast, doxorubicin exhibited the highest potency but showed significant toxicity toward normal fibroblasts, highlighting its lack of selectivity.

The higher SIs of the acetone extract suggest its potential as a safer anticancer agent, offering effective tumor inhibition with reduced toxicity to normal cells. These results emphasize the need for further investigation into the bioactive compounds responsible for cytotoxicity and their underlying mechanisms of action. Future studies should focus on isolating key phytochemicals, exploring their molecular interactions, and evaluating their efficacy in in vivo models to assess their therapeutic potential.

In a comparative analysis to the present study, an investigation conducted in Saudi Arabia, the essential oil derived from *R. chalepensis* exhibited a limited inhibitory effect on the PC-3 (prostate cancer) cell line, while demonstrating a significant inhibitory impact on the A2780 (ovarian cancer) cell line [34]. The methanolic extract of *R. chalepensis* demonstrated cytotoxic effects on HEP-G2 and L5178Y-R cells, while showing no impact on normal VERO, PBMC, and J774A.1 cells [15].

A subsequent study demonstrated that the essential oil derived from *R. chalepensis* displayed anticancer effects across multiple cancer cell lines, such as HEK293, NCI-N87, K562, MIA-Paca2, U2OS, U87-MG, JIMT-T1, MRC-5, A549, and PC3 [35]. The oil extract exhibited cytotoxicity against MCF-7, T47D, and Caco-2 cancer cell lines in a dose-dependent manner [12].

5. Conclusion

The phytochemical study of *R. chalepensis* extracts demonstrated varying contents according to the solvent used. The acetone extract showed a high concentration of monoterpenes, flavonoids, and alkaloids with 4-thujene (8.9%) and sophoretin (8%) as the primary components. The aqueous extract was rich in rutamarin (11.4%), while fatty acids (20.4%) and phenylpropenes (14.9%) dominated the hexane extract. Furthermore, the acetone extract demonstrated the most potent effects and higher selectivity for cancer cells, while the aqueous and hexane extracts showed moderate activity. The results indicate that the acetone extract had considerable potential as an anticancer agent with minimal damage to normal cells. Future studies should focus on clarifying the mechanism of action of the acetone extract, exploring its potential in combinatorial medicines, and conducting in vivo studies to confirm its therapeutic efficacy and safety.

Data Availability Statement

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

Consent

The authors have nothing to report.

Disclosure

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Mohammad Alwahsh conceptualized the study. Mohammad Alwahsh, Wassan Jarrar, and Arwa R. Althaher designed the experiments. Arwa R. Althaher, Mohammad Alwahsh, Wassan Jarrar, and Aya Hasan conducted laboratory experiments. Arwa R. Althaher and Mohammad Alwahsh analyzed the data. Arwa R. Althaher, Mohammad Alwahsh, and Wassan Jarrar wrote the manuscript. All authors revised the manuscript and read and approved the final submission.

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References

[1] J. Boutry, S. Tissot, B. Ujvari, et al., "The Evolution and Ecology of Benign Tumors," *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer* 1877, no. 1 (2022): 188643, https://doi.org/10.1016/j.bbcan.2021.188643.

[2] World Health Organization, *Global Cancer Burden Growing* (Amidst Mounting Need for Services, 2024), https://www.who.int/news/item/01-02-2024-global-cancer-burden-growing--amidst-mounting-need-for-services.

[3] F. Bray, M. Laversanne, H. Sung, et al., "Global Cancer Statistics 2022: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries," *CA: A Cancer Journal for Clinicians* 74, no. 3 (2024): 229–263, https://doi.org/10.3322/caac.21834.

[4] A. Zafar, M. Arshad, S. Khan, A. Nawaz, A. W. Khan, and A. Latif, "Advancements and Limitations in Traditional Anti-Cancer Therapies: A Comprehensive Review of Surgery, Chemotherapy, Radiation Therapy, and Hormonal Therapy," *Frontiers in Oncology* 16, no. 1 (2025): 607.

[5] F. R. Weth, A. Müller, Y. Zhang, and T. Hanke, "Unlocking Hidden Potential: Advancements, Approaches, and Obstacles in Repurposing Drugs for Cancer Therapy," *Pharmacological Research* 130, no. 5 (2024): 703–715.

[6] M.-Y. Li, A. Gu, J. Li, et al., "Exploring Food and Medicine Homology: Potential Implications for Cancer Treatment Innovations," *Acta Materia Medica* 4, no. 2 (2025): 200–206, https://doi.org/10.15212/amm-2025-0003.

[7] C. A. Dehelean, I. Marcovici, C. Soica, et al., "Plant-Derived Anticancer Compounds as New Perspectives in Drug Discovery and Alternative Therapy," *Molecules* 26, no. 4 (2021): 1109, https://doi.org/10.3390/molecules26041109.

[8] N. H. Aboalhaija, H. Syaj, F. Afifi, S. Sunoqrot, E. Al-Shalabi, and W. Talib, "Chemical Evaluation, In Vitro and In Vivo

Anticancer Activity of Lavandula Angustifolia Grown in Jordan," *Molecules* 27, no. 18 (2022): 5910, https://doi.org/10.3390/molecules27185910.

[9] N. Aboalhaija, A. Alsalman, S. Alabed, W. Talib, I. Abaza, and F. Afifi, "Phytochemical Screening and Cytotoxic Evaluation of Salvia Greggii Extracts: An In Vitro and In Silico Study," *Farmacia* 72, no. 1 (2024): 171–177, https://doi.org/10.31925/farmacia.2024.1.18.

[10] Y. Liu, F. Zhao, Q. Wang, Q. Zhao, G. Hou, and Q. Meng, "Current Perspectives on Paclitaxel: Focus on Its Production, Delivery and Combination Therapy," *Mini Reviews in Medicinal Chemistry* 23, no. 18 (2023): 1780–1796, https://doi.org/10.2174/1389557523666230210145150.

[11] Radha, N. Kumari, S. Prakash, et al., "Medicinal and Aromatic Plants as Potential Sources of Bioactives Along With Health-Promoting Activities," *Current Food Science and Technology Reports* 2, no. 4 (2024): 359–376, https://doi.org/10.1007/s43555-024-00042-8.

[12] A. R. Althaher, S. A. Oran, and Y. K. Bustanji, "Phytochemical Analysis, In Vitro Assessment of Antioxidant Properties and Cytotoxic Potential of Ruta Chalepensis L. Essential Oil," *Journal of Essential Oil Bearing Plants* 23, no. 6 (2020): 1409–1421, https://doi.org/10.1080/0972060x.2020.1871078.

[13] H. Ali and A. Q. Abdulwahab, "Potential of Some Therapeutic Effect of Ruta Graveolenes Plant and Their Bioactivities: A Review," *Medical Journal of Ahl Al-Bayt University* 3, no. 1 (2024): 174–189.

[14] H. Ghazghazi, C. Aouadhi, M. Weslati, F. Trakhna, A. Maaroufi, and B. Hasnaoui, "Chemical Composition of Ruta Chalepensis Leaves Essential Oil and Variation in Biological Activities Between Leaves, Stems and Roots Methanolic Extracts," *Journal of Essential Oil Bearing Plants* 18, no. 3 (2015): 570–581, https://doi.org/10.1080/0972060x.2014.905757.

[15] J. H. Elizondo-Luévano, N. E. Rodríguez-Garza, A. F. Bazaldúa-Rodríguez, et al., "Cytotoxic, Anti-Hemolytic, and Antioxidant Activities of Ruta Chalepensis L. (Rutaceae) Extract, Fractions, and Isolated Compounds," *Plants* 12, no. 11 (2023): 2203, https://doi.org/10.3390/plants12112203.

[16] A. R. Althaher, S. A. Oran, and Y. K. Bustanji, "Induction of Apoptosis by Ruta Chalepensis L. Essential Oil in Human Breast Cancer Cells (MCF-7)," *Journal of Pharmacy & Pharmacognosy Research* 10, no. 1 (2022): 73–83, https://doi.org/10.56499/jppres21.1180_10.1.73.

[17] A. R. Althaher, S. A. Oran, M. W. Awadallah, et al., "Chemical Composition, Antioxidant, and Antibacterial Activity of Ruta Chalepensis L. Ethanolic Extract," *Chemistry and Biodiversity* 21, no. 4 (2024): e202400026, https://doi.org/10.1002/cbdv.202400026.

[18] S. H. Altayeb, F. A. Khleefa, S. I. Yagoob, et al., "Antimicrobial, Phytochemical Screening and GC-MS Analysis of Ruta Chalepensis L. Leaves and Stems Extracts," *Pharmacognosy Research* 16, no. 4 (2024): 985–991, https://doi.org/10.5530/pres.16.4.112.

[19] J. E. Lee, J. T. M. Jayakody, J. I. Kim, et al., "The Influence of Solvent Choice on the Extraction of Bioactive Compounds From Asteraceae: A Comparative Review," *Foods* 13, no. 19 (2024): 3151, https://doi.org/10.3390/foods13193151.

[20] K. A. Shadid, A. K. Shakya, R. R. Naik, et al., "Phenolic Content and Antioxidant and Antimicrobial Activities of Malva Sylvestris L., Malva Oxyloba Boiss., Malva Parviflora L., and Malva Aegyptia L. Leaves Extract," *Journal of Chemistry* 2021, no. 1 (2021): 1–10, https://doi.org/10.1155/2021/8867400.

[21] M. Alwahsh, A. Al-Doridee, S. Jasim, O. Awwad, R. Hergenröder, and L. Hamadneh, "Cytotoxic and Molecular Differences of Anticancer Agents on 2D and 3D Cell Culture," *Molecular Biology Reports* 51, no. 1 (2024): 721, https://doi.org/10.1007/s11033-024-09669-1.

[22] T. Widiandani, T. Tandian, B. D. Zufar, et al., "In Vitro Study of Pinostrobin Propionate and Pinostrobin Butyrate: Cytotoxic Activity Against Breast Cancer Cell T47D and Its Selectivity Index," *Journal of Public Health in Africa* 14, no. 1 (2023): 2516, https://doi.org/10.4081/jphia.2023.2516.

[23] P. C. Situmorang, S. Ilyas, S. E. Nugraha, R. A. Syahputra, and N. M. A. Nik Abd Rahman, "Prospects of Compounds of Herbal Plants as Anticancer Agents: A Comprehensive Review From Molecular Pathways," *Frontiers in Pharmacology* 15 (2024): 1387866, https://doi.org/10.3389/fphar.2024.1387866.

[24] P. Pant, S. Pandey, and S. Dall'Acqua, "The Influence of Environmental Conditions on Secondary Metabolites in Medicinal Plants: A Literature Review," *Chemistry and Biodiversity* 18, no. 11 (2021): e2100345, https://doi.org/10.1002/cbdv.202100345.

[25] M. S. S. Khan and M. G. Dashti, "Optimizing Extraction Techniques for Maximized Bioactive Compound Yield in Medicinal Herbs," *Australian Herbal Insight* 6, no. 1 (2023): 1–10.

[26] C. M. Santos Junior, S. M. C. Silva, E. M. Sales, et al., "Coumarins From Rutaceae: Chemical Diversity and Biological Activities," *Fitoterapia* 168 (2023): 105489, https://doi.org/10.1016/j.fitote.2023.105489.

[27] V. F. Otvagin, N. S. Kuzmina, E. S. Kudriashova, A. V. Nyuchev, A. E. Gavryushin, and A. Y. Fedorov, "Conjugates of Porphyrinoid-Based Photosensitizers With Cytotoxic Drugs: Current Progress and Future Directions Toward Selective Photodynamic Therapy," *Journal of Medicinal Chemistry* 65, no. 3 (2022): 1695–1734, https://doi.org/10.1021/acs.jmedchem.1c01953.

[28] T. X. Chen, S. K. Wang, Y. Q. Zhang, et al., "7, 8-Dihydroxyflavone Displayed Antioxidant Effect Through Activating HO-1 Expression and Inhibiting Caspase-3/PARP Activation in RAW264. 7 Cells," *Journal of Biochemical and Molecular Toxicology* 38, no. 1 (2024): e23602, https://doi.org/10.1002/jbt.23602.

[29] V. T. Alexa, A. Galuscan, C. M. Soica, et al., "In Vitro Assessment of the Cytotoxic and Antiproliferative Profile of Natural Preparations Containing Bergamot, Orange and Clove Essential Oils," *Molecules* 27, no. 3 (2022): 990, https://doi.org/10.3390/molecules27030990.

[30] K. Bhattacharya, S. Mahato, S. Deka, N. R. Chanu, A. K. Shrivastava, and P. Khanal, "Netting Into the Sophoretin Pool: An Approach to Trace GSTP1 Inhibitors for Reversing Chemoresistance," *Computational Biology and Chemistry* 108 (2024): 107981, https://doi.org/10.1016/j.compbiolchem.2023.107981.

[31] R. Bruni, D. Barreca, M. Protti, et al., "Botanical Sources, Chemistry, Analysis, and Biological Activity of Furanocoumarins of Pharmaceutical Interest," *Molecules* 24, no. 11 (2019): 2163, https://doi.org/10.3390/molecules24112163.

[32] J. S. Kim, D. K. Kim, J. Y. Moon, M. Y. Lee, and S. K. Cho, "Oleic Acid Inhibits the Migration and Invasion of Breast Cancer Cells With Stemness Characteristics Through Oxidative Stress-Mediated Attenuation of the FAK/AKT/NF-κB Pathway," *Journal of Functional Foods* 116 (2024): 106224, https://doi.org/10.1016/j.jff.2024.106224.

[33] S. Zhu, W. Jiao, Y. Xu, et al., "Palmitic Acid Inhibits Prostate Cancer Cell Proliferation and Metastasis by Suppressing the PI3K/Akt Pathway," *Life Sciences* 286 (2021): 120046, https://doi.org/10.1016/j.lfs.2021.120046.

[34] L. M. Al Masoudi, H. Hamdi, and A. A. Zaid, "Chemical Composition, Antifungal, Antibacterial, Antioxidant, Antiviral and Cytotoxic Activities of Ruta Chalepensis L. Fixed Oil From Saudi Arabia," *Journal of Biobased Materials and Bioenergy* 18, no. 4 (2024): 701–709, https://doi.org/10.1166/jbmb.2024.2414.

[35] F. M. Abdoul-Latif, A. Elmi, A. Merito, et al., "Essential Oil of Ruta Chalepensis L. From Djibouti: Chemical Analysis and Modeling of In Vitro Anticancer Profiling," *Separations* 9, no. 12 (2022): 387, https://doi.org/10.3390/separations9120387.