RESEARCH ARTICLE

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In Vitro Evaluate the Antiproliferative Impact of *Cnicus Benedictus* L. Leaves Methanolic Extract on Cervical Cancer

Diyar M Jalil[1], Tiba Th Al-Mahdawi[2], Murooj G Jameel[1]*, Mayada Talal[1]

Abstract

Objective: The present study aimed to evaluate the efficacy of the Cnicus benedictus leaf methanolic extract (CBHE) in reducing the growth of cervical cancer cells (Hela cancer cell line). **Methods:** The extraction was achieved using the Soxhlet apparatus. The study utilized a human cervical cancer cell line for antiproliferative evaluation and a human fibroblast cell line for toxicity assessment on normal cells. The incubation periods were 24 and 72 hours, and the concentration of the extract varied between 0.1 and 1,000 µg/ml. **Results:** The study exhibits that the methanolic extract of *Cnicus Benedictus* leaves can lessen the growth of human cervical cancer cells. The growth inhibition of the extract was dependent on the concentration and time, with the highest inhibition rate seen at 1,000 µg/ml after 72 hours of incubation. The study also revealed that the extract had minimal impact on the growth of normal cells. **Conclusion:** The study shows that *Cnicus Benedictus* leaves methanolic extract has ability to inhibit the growth of human cervical cancer cells *in vitro*. The extract cytotoxic behavior was (cell cycle and cell non-cycle) specific. The research also found that the extract selectively kills cancer cells rather than normal cells, indicating its safety in their effectivity.

Keywords: *Cnicus Benedictus* L. leaves- Hela cancer cell line- cervical cancer cell line- Iraqi blessed thistle

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Introduction

Globally, cancer is responsible for 12.5% of all fatalities. The mortality rate attributed to cancer exceeds the combined mortality rate attributed to tuberculosis, ADIS, and malaria [1]. Abnormal cellular proliferation is the hallmark of cancer, a complicated multifactorial cell disease. The cellular accumulation of different genetic and epigenetic processes is necessary for the development and progression of cancer [2].

Cervical cancer, or CC, is a type of cancer that typically affects women in developing and impoverished nations. Untreated cases of CC cause an increase in morbidity and death among women. According to the epidemiology of CC, early detection at an early stage can help minimize the progression of the neoplastic disease, or the application of specific combat and operational risk factors of various variables connected with the formation/development of CC could help avoid being infected [3].

Cervical cancer is a predominant malignancy that mainly concerns women globally, with an annual incidence of around 500,000 new cases. It is classified as the second most prevalent illness, ranking behind breast cancer [4]. Since natural phenolic compounds are the main components of fruits and vegetables and are also necessary for a healthy diet, they have acquired a particularly crucial role in the prevention of cancer along with additional plant extracts and therapeutic plants. Their findings regarding the inhibition of the proliferation of multiple cancer cell lines have also been intriguing and have the potential to be investigated further as anticancer medicines [5].

Thistle-like medicinal plant of the Asteraceae family is *Cnicus benedictus* L., also known as "blessed thistle." It is the only species in the genus Cnicus. The plant is an annual that has leathery leaves. *Cnicus Benedictus* L. is generally found in the northern Iraqi cities. *Cnicus Benedictus* L has considerable antioxidant and anticancer effects and activates depending on geographic area and production process [6, 7, 8]. Several previous studies discovered that *Cnicus Benedictus* L antioxidant abilities are controlled by environmental factors such as growth duration and area [6, 9, 10].

Multiple studies exhibit that C. Benedictus L. has scavenging activity against free radicals and nitrites. These studies suggest that blessed thistle phenolic and flavonoid constituents may improve its overall effectiveness due to its antioxidant properties [11]. This study shows that blessed thistle anticancer abilities are mainly related to its phytochemical components, particularly flavonoids and glycosides [12]. Several studies were done to assess the anticancer abilities of Blessed Thistle. One of these studies investigated the ethanol-based extract derived from the plant of *Cnicus Benedictus* L. result outcomes show that the extract effectively eradicates AMN-3 mammary

*[1]Department of Pharmacy, Al-Yarmok University College, Diyala, Iraq. [2]Department of Pharmacy, Bilad Alrafidain University College, Diyala, Iraq. *For Correspondence: Muroojgazi@al-yarmok.edu.iq*

adenocarcinoma cancer cells, with a cytotoxic pattern depending on each concentration and time [10, 13].

Several studies were conducted on the same subject as the present study; However, these findings were not included in the analysis of the *Cnicus Benedictus* leaves. The aim of the study was to determine the growth inhibition capacity of a methanolic extract of *Cnicus Benedictus* leaves on cervical cancer cell lines and human fibroblast cell lines.

Materials and Methods

Natural product characteristics

The leaves of (*Cnicus Benedictus* L.) were collected for the study from the highest mountain in the north of Iraq during the period between May and July, 2023. A specimen was retained in the herbarium of the Faculty of Sciences at the University of Baghdad for identification.

By mashing the plant leaves into a fine powder, a concentrated alcoholic solution was acquired by engaging 100 grams of the powder in 1000 ml of 70% methanol

Materials and reagents

Thiazolyl blue tetrazolium bromide (MTT) (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide stain) , Zinc-Hcl, Alkaline reagent were purchased from Sigma-Aldrich (USA). Roswell Park Media Institute (RPMI) 1640, penicillin/streptomycin, trypsin-EDTA and fetal bovine serum mycoplex (FBS) were obtained from tissue culture/ Iraqi Center for Cancer and Medical Genetics Research (ICCMGR). The methanol (MeOH) was used were of the highest purity (UK).

Analytical balance was used for weighed leaves of the plant. Sieve was used for separate fine particles from plant powder. Filter paper (whattman) was used for filtration process.

During the soxhlet extraction process, the solution was heated using a heater mantle. For measuring and preparing the solutions, 100 mL volumetric flasks and conical flasks were utilized. Burettes, beakers, and pipettes were used in the standard titration process. Flavonoids, alkaloids, carbohydrates, phenolic compounds, tannins, terpenoids, coumarin, and lignin, were scrutinized employing several detector reagents [10].

In vitro model system
Cell culture

The human fibroblast and Hela cancer cell line were obtained (ICCMGR); the cells were cultured in 75 cm2 tissue culture flasks. It is a regulated environment with a 37°C temperature and a humid atmosphere that contains 5% carbon dioxide. The RPMI-1640 medium was provided by Sigma Chemicals (UK) with 10% of bovine calf serum (FBS), the media also contained (100 g/mL of streptomycin and 100 U/mL) 1% of penicillin-streptomycin complex [14]. And the protocols of this work were approved by ethics committee of scientific committee of ICCMGR.

Design and outcome of experiment
Anti-proliferation assay (MTT)

4-2-(5-bromo-1H-indol-2-carbonyl)-N-(4-methoxyphenyl) hydrazine-1-carbothioamide was serially diluted and applied to the cells for twenty-four hours, followed by 3-(4, 5-dimethylthiazol-2-yl) and so on.2-Phosphate Buffer Saline (PBS) was used to generate a 5 mg/ml solution of MTT (diphenyltetrazolium bromide salt). Following the plate's incubation, ELIZA measured the absorbance at 550 nanometers [8].

Assessment of growth inhibition

Cells were grown on 96-well microtiter plates for the investigation. The concentration of ethanolic extract emanating from blessed thistle leaves were applied to the cells. Throughout the logarithmic expansion phase, the population of cancer cells per well expanded steadily throughout dual incubation days. The plant extract's inhibitory impacts will be assessed for cell multiplication. Each well must have seven thousand cells, which is crucial.

Cancer cells were exposed to a culture medium supplemented with 10% calf serum. The plates were incubated at 37°C for 24 hours to improve the adherence of cancer cells. Afterward, a maintenance medium was employed to create a series of dilutions for the plant extract, including quantities varying from 0.1 to 1,000 µg/ml [15].

After incubating for 24 hours, the cells were exposed to a six cope of each concentration, with a volume of 200µl for each cope. 200µl of serum-free media were added to the control wells, and the research plates were incubated for two time periods: twenty-four hours and seventy-two hours. After applying a self-adhesive substance, the plates were placed inside the incubator. After the incubation period, the activity of the treatments was evaluated by staining the treated and control samples with MTT dye

The optical density of every well was determined using an ELISA reader that used a 550 nm transmission wavelength; a mathematical computation was employed to determine the growth inhibition rate; the equation gives the result [16-18].

$$\text{Growth inhibition \%} = \frac{\text{Optical density of control wells - Optical density of treated wells}}{\text{Optical density of control wells}} \times 100\%$$

Statistical analysis of data

In order to determine how each element affected the study's parameters, The Statistical Analysis System (SAS) with SPSS version 16 software was employed; to find out whether there were any statistically significant differences among means, they used the t-test and LSD. Statistical significance was considered at $p < 0.05$.

Research ethics

There was no study about humans involved in the investigation

Results

Cnicus Benedictus L. leaves contain multiple types of phytochemicals, such as tannins, flavonoids, carbohydrates, phenolic compounds, terpenoids, coumarins, and lignins. As shown in Table 1.

The presence of these phytochemicals in the plant leaves plays a role in plant antioxidant, anticancer, and antibacterial properties [11, 19, 20]. The study exhibited a significant increase in inhibiting cervical cancer cell growth when utilizing larger concentrations of plant extract, especially 1,000 µg/ml, and longer incubation times, especially 72 hrs. The growth inhibition exhibited a noticeable pattern that was affected by the concentration and length of treatment. Table 2 and Figure 1. The impact of concentration of plant extracts from the leaves of *Cnicus Benedictus* L. and incubation course on the expansion of normal fibroblast cells as shown in Table 3

Table 1. Phytochemical Components in the Methanolic Extract of Cnicus Benedictus Leaves

Detection of the Phytochemical		
outcome	Reagents	Phytochemicals type
Positive	Mayer’s, Wagner’s	Alkaloids
Positive	Borntrager’s test, Legal’s test	glycoside
Positive	Trim-Hill, Liebermann Burchard's	Terpenoids
Positive	Zinc-Hcl, Alkaline reagent, Ferric chloride, Lead-acetate	Flavonoids
Positive	Ferric chloride	tannins
Positive	Ferric chloride	Phenolic
Positive	Molish’s test	carbohydrate
Negative	Ninhydrin test	Protein and amino acid

Table 2. The Impact of Diver’s Concentration of Plant Extracts and Incubation Course on the Expansion of Cervical Cancer Cells

Plant extract concentration	Incubation times		P-value
	Twenty-four	Seventy-two	
0.1	3 C	11 C	0.001*
1	6 C	20 C	0.014*
10	22 B	36 B	0.0001*
100	33 B	45 B	0.023*
1000	50 A	71 A	0.002*
LSD	11.28	11.84	-
IC 50	665.8 µg/ml	943.3 µg/ml	-

Significant differences (P< 0.05) were denoted by diverse capital letters between the means of the relevant columns; *, Significant at (P≤0.05), (N. S; Non- Significant).

Table 3. The Impact of the Diver’s Concentration of Plant Extracts and Incubation Course on the Expansion of Normal Fibroblast Cells

Plant extract concentration	Incubation times		p-value
	Twenty-four	Seventy-two	
0.1	12:00 AM	1 B	0.116 N. S
1	2:00 AM	2 B	1.000 N. S
10	3:00 AM	1 B	0.158 N. S
100	5:00 AM	7:00 AM	0.196 N. S
1000	3:00 AM	5 AB	0.700 N. S
LSD	4.3	4.3	-
IC 50	57.3 µg/ml	45.1 µg/ml	-

Significant differences (P< 0.05) were denoted by diverse capital letters between the means of the relevant columns; *, Significant at (P≤0.05), (N. S; Non- Significant).

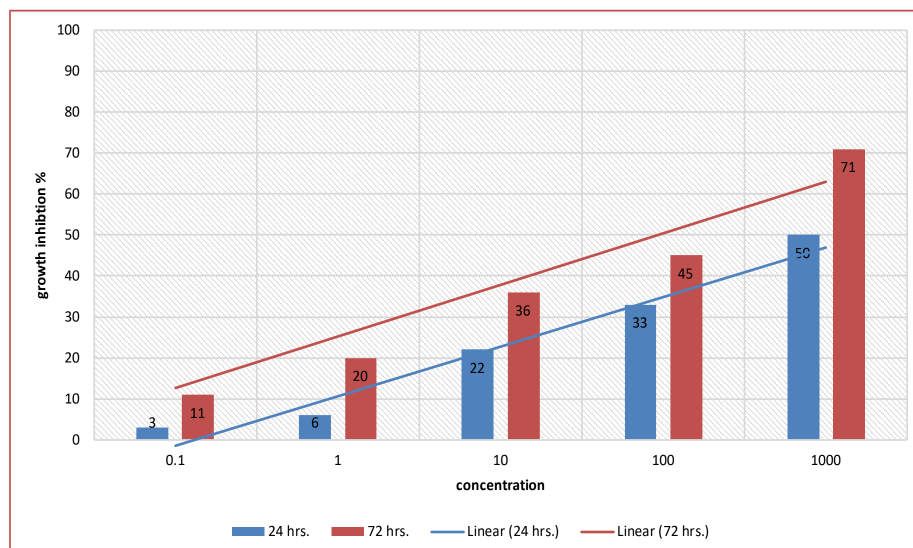

Figure 1. The Impact of Concentration of Plant Extracts and Incubation Course on the Expansion of Cervical Cancer Cells

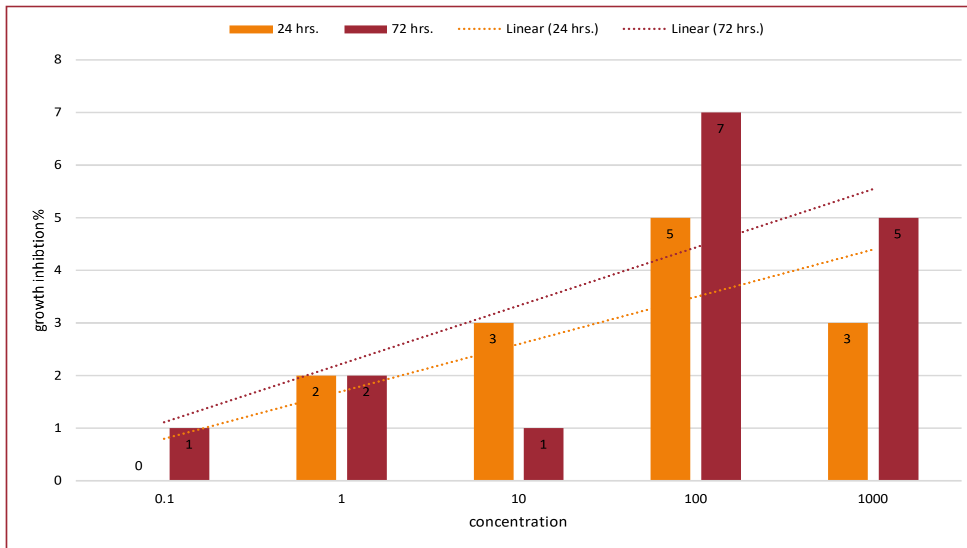

Figure 2. The Impact of Concentration of Plant Extracts and Incubation Course on the Expansion of Normal Fibroblast Cells

Table 4. The Effects of Various Plants Extract Concentrations on the Growth of Embryo Fibroblast and HeLa Cell Lines Over the Course of 24 hours.

Concentration	Cell line		p-value
	Hela	embryo fibroblast	
0.1	3 C	12:00 AM	0.007*
1	6 C	2:00 AM	0.094 N. S
10	22 B	3:00 AM	0.0001*
100	33 B	5:00 AM	0.0001*
1000	50 A	3:00 AM	0.0001*
LSD	11.28	4.3	-
IC 50	665.8 µg/ml	57.3 µg/ml	-

Significant differences (P< 0.05) were denoted by diverse capital letters between the means of the relevant columns. *, Significant at (P≤0.05), (N. S; Non- Significant).

and Figure 2.

The effects of various plant extract concentrations on the growth of embryo fibroblast and Hela cell lines over the course of 24 and 27 hours respectively as shown in

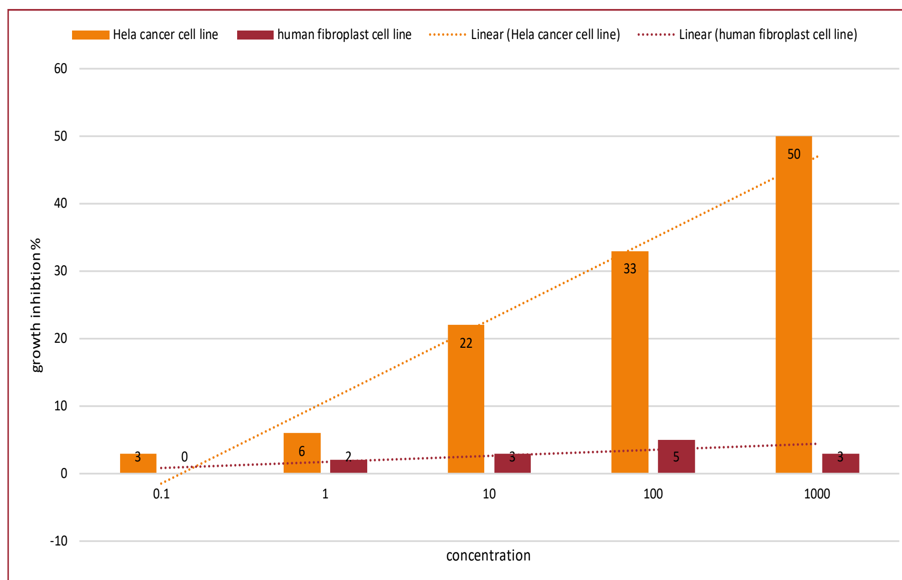

Figure 3. The Effects of Various Plant Extract Concentrations on the Growth of Embryo Fibroblast and Hela Cell Lines Over the Course of 24 hours.

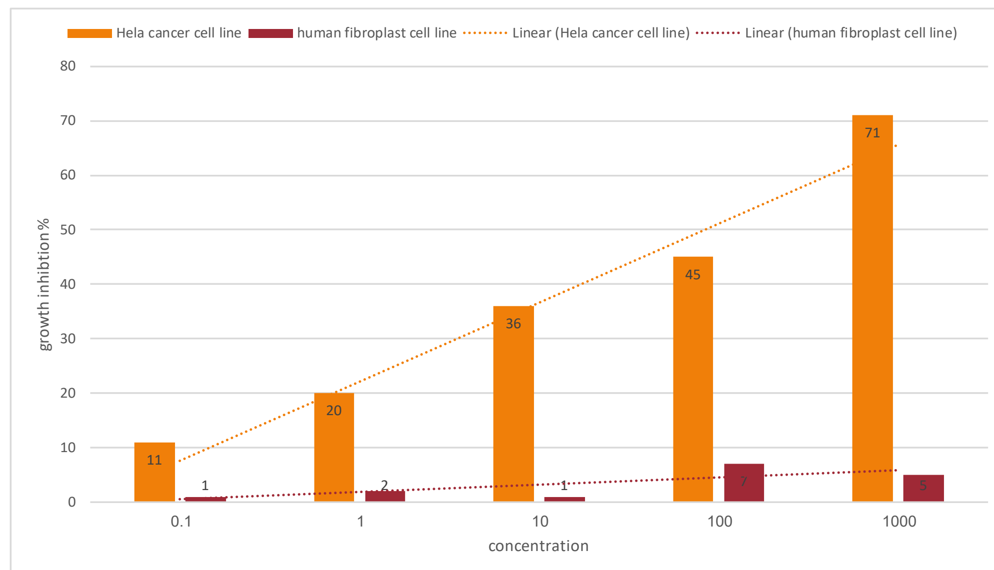

Figure 4.The Effects of Various Plant Extract Concentrations on the Growth of Embryo Fibroblast and Hela Cell Lines Over the Course of 27 hours.

Table 5. The Effects of Various Plants Extract Concentrations on the Growth of Embryo Fibroblast and HeLa Cell Lines Over the Course of 27 hours.

Concentration	Cell line		p-value
	Hela	embryo fibroblast	
0.1	11 C	1 B	0.0001*
1	20 C	2 B	0.004*
10	36 B	1 B	0.0001*
100	45 B	7:00 AM	0.0001*
1000	71 A	5 AB	0.0001*
LSD	11.84	4.3	
IC 50	943.3 µg/ml	45.1 µg/ml	

Significant differences ($P< 0.05$) were denoted by diverse capital letters between the means of the relevant columns; *, Significant at ($P\leq 0.05$), (N. S; Non- Significant).

Tables (4 and 5) and Figures (3 and 4). The study outcomes indicate that the plant extract has a slight impact on the growth of normal human fibroblast cells, as noticed by its cytotoxic effects. However, a considerable difference was seen when comparing the impact of the plant extract on Hela cells and human fibroblast cells (Figure 5).

Discussion

The results of present study are compatible with prior research to evaluate the anticancer impacts of blessed thistle; one of these studies shows the capability of blessed thistle flowers to lessen the growth of mouse mammary adenocarcinomas *in vitro* [11]. While the other one indicates the ability of blessed thistle roots to hinder the proliferation of Dalton's lymphoma ascites cells.

The anticancer activities of plant extracts are mainly attributed to their ingredients, specifically phytochemicals and cnicin. Cnicin showed an ability to lessen the growth of diverse cancer cell lines, as pig kidney epithelial ($LLC-PK_{11}$), human malignant melanoma (SK-MEL), human ductal carcinoma (BT-549), and primary myeloma cell line. Whereas stromal cells and endothelial cells were unaffected, the mechanism of cnicin cytotoxicity can be related to the activation of caspases, accumulation of reactive oxygen species, and downregulation of nuclear factor kappa-light-chain-

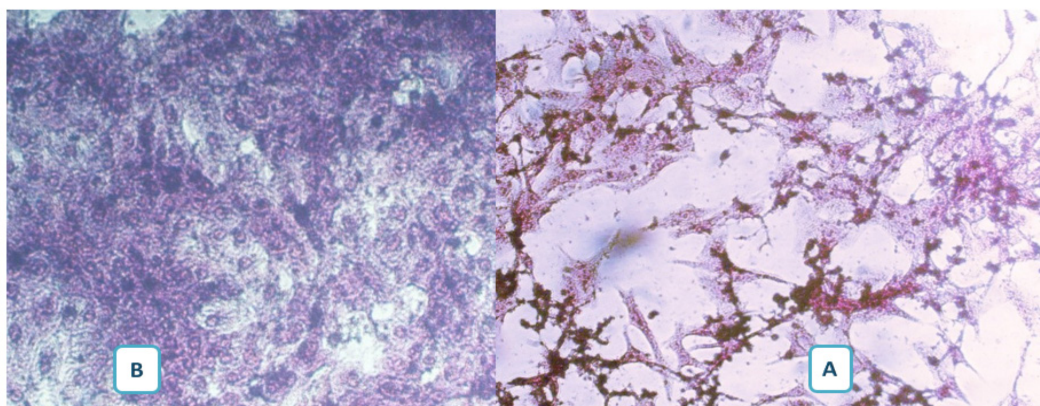

Figure 5. HeLa Cells Morphology. (A) cells after being treated with 10000 (µg/ml) of (methanolic extract Cnicus Benedictus L. leaves) at 72hr, (B) HeLa cells of the control group.

enhancer of activated B cell [21-24].

Flavonoids have an inhibitory effect on the multiplication of some types of cancer cells. Research has shown that the effect of flavonoids is more significant in cells with a high mitotic index than in cells with a lower mitotic Index [25, 26].

The existence of terpenoid substance in *Cnicus Benedictus* has been shown to contribute to its anticancer effects. These substances generate autophagy by activating signaling pathways and reactive oxygen species. It is guiding to the total elimination of malignant cells [27, 28]. In addition, tannins improve the plant's anticancer effects by interrupting the replication of cellular DNA and inhibiting the growth of cancer cells [3, 14, 29].

The methanolic extract of blessed thistle leaves is thought to have anticancer effects attributed to its consequence on osmolality. Extracts include proteins, carbohydrates, minerals, and other compounds that generate a hypertonic condition in cancer cells. Consequently, the cancer cells are exposed to an inimical condition, potentially conducting an osmotic shock that changes in intensity depending on the plant extract concentration [30-32].

One limitation of the study was the evaluation of the plant extract cytotoxicity non-involved in a 48-hour incubation period. This time has not supplied any information on the pattern of plant cytotoxicity, unlike the 24 and 72-hour incubation periods. In conclusion the study demonstrated the ability of the methanolic extract from *Cnicus Benedictus* leaves to hinder the proliferation of cervical cancer cells. The extract's cytotoxic effects were both cycle-specific and non-cycle-specific patterns. Additionally, the study indicated that the extract exhibits selective toxicity towards cancer cells as opposed to normal cells, suggesting a high level of safety for the plant extract.

Author Contribution Statement

All authors contributed equally in this study.

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Abbreviations

(ICCMGR): The Iraqi Centre for Cancer and Medical Genetics Research.

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide stain

RPMI: Roswell park memorial institute medium

SAS: Statistical Analysis System

LSD: Least Significant Difference

Availability of data

All data can be requested from the author

Data analysis and interpretation

Composition of the article,

Reviewing the essay critically for key conceptual points, Proficiency in statistical analysis, Ultimate endorsement and guarantee of the article.

Conflicts of interest

There is no conflict of interest.

References

1. Deo SVS, Sharma J, Kumar S. GLOBOCAN 2020 report on global cancer burden: challenges and opportunities for surgical oncologists. Ann Surg Oncol. 2022;29(11):6497-6500. https://doi.org/10.1245/s10434-022-12151-6.
2. Rock CL, Thomson C, Gansler T, Gapstur SM, McCullough ML, Patel A, et al. American Cancer Society guideline for diet and physical activity for cancer prevention. CA Cancer J Clin. 2020;70(4):245-271. https://doi.org/10.3322/caac.21591.
3. Riaz L, Manazir S, Jawed F, Ali SA, Riaz R. Knowledge, Perception, and Prevention Practices Related to Human Papillomavirus-based Cervical Cancer and Its Socioeconomic Correlates Among Women in Karachi, Pakistan. Cureus. 2020;12(3):e7183. https://doi.org/10.7759/cureus.718.
4. Omone OM, Lucky K, Kozlovszky M. Effective Prevention of High-Risk Cervical Cancer among Women in Developing Countries: A Case-Study in Nigeria. Int J Health Life Sci. 2020;6(3):01-22. https://doi.org/10.20319/lijhls.2020.63.0122.
5. Roleira FM, Varela CL, Costa SC, Tavares-da-Silva EJ. Phenolic derivatives from medicinal herbs and plant extracts: anticancer effects and synthetic approaches to modulate biological activity. Studies in Natural Products Chemistry. 2018;57:115-156. https://doi.org/10.1016/B978-0-444-64057-4.00004-1
6. Allawi MM, Mahmood AAR, Tahtamouni LH, AlSakhen MF, Kanaan SI, Saleh KM, et al. New indole-6-carboxylic acid derivatives as multi-target antiproliferative agents: Synthesis, in silico studies, and cytotoxicity evaluation. Chem Biodivers. 2024;21(2):e202301892. https://doi.org/10.1002/cbdv.202301892.
7. Batziakas KG, Stanley H, Batziakas AG, Brecht JK, Rivard CL, Pliakon ED. Reducing postharvest food losses in organic spinach with the implementation of high tunnel production systems. Agron Sustai Dev. 2020;40:42. https://doi.org/10.1007/s13593-020-00648-x.
8. Wied JP, Perotto-Baldivieso HL, Conkey AA, Brennan LA, Mata JM. Invasive grasses in South Texas rangelands: historical perspectives and future directions. Invas Plant Sci Mana. 2020;13(2):41-58. https://doi.org/10.1017/inp.2020.11
9. Jian YL, Lee YH, Huang JD, Ho CT, Lou SN. Flavonoid Compositions and Antioxidant Activity of Immature Kumquat in Different Flowering Periods. Taiwan J Agric Chem Food Sci. 2020;58(1). https://doi.org/10.6578/TJACFS.202002_58(1).0004
10. Ionescu N, Badea O, Podea M, Popescu D, Ghiorghe C, Nicolaie M, et al. Mating dioecious system of cirsium arvense (L.) Scop. Weed. Curr Trends Nat Sci. 2020;9(17):38-51. https://doi.org/10.47068/ctns.2020.v9i17.005.
11. Yasin Al-Samarray YS, Jumaa AH, Hashim WS, Khudhair YI. The Cytotoxic Effect Of Ethanolic Extract Of Cnicus

Benedictus L. Flowers On The Murine Mammary Adenocarcinoma Cancer Cell Lineamn-3. Biochem Cell Arch. 2020;20(2):4345-4348.
12. Salim NS, Abdel-Alim M, Said HE, Foda MF. Phenolic Profiles, Antihyperglycemic, Anti-Diabetic, and Antioxidant Properties of Egyptian Sonchus oleraceus Leaves Extract: An In Vivo Study. Molecules. 2023;28(17):6389. https://doi.org/10.3390/molecules28176389.
13. Rezig K, Benkaci-Ali F, Foucaunier ML, Laurent S, Umar HI, Alex OD, Tata S. HPLC/ESI-MS Characterization of Phenolic Compounds from Cnicus benedictus L. Roots: A Study of Antioxidant, Antibacterial, Anti-inflammatory, and Anti-Alzheimer's Activity. Chem Biodiversity. 2024;21(1):e202300724. https://doi.org/10.1002/cbdv.202300724. Epub 2023 Dec 19
14. Yasin YS, Jumaa, AH, Jabbar S, Abdulkareem AH. Effect of Laetrile Vinblastine Combination on the Proliferation of the Hela Cancer Cell Line. Asian Pac J Cancer Prev. 2023;24(12):4329-4337. https://doi.org/10.31557/APJCP.2023.24.12.4329.
15. Rahangdale P, Wankhade AM. Ganoderma lucidum ethanolic extract for the treatment of androgenic alopecia in rats with testosterone-induced Baldness. Med Pharm J. 2023;2(2):107–20. https://doi.org/10.55940/medphar202343.
16. Gasmi M, Rahma Z, Williams-Hooker RW, Sharma A. Research Article Production and Characterization of Recombinant Multi-Epitope G Glycoprotein of Rabies Virus. Med Pharm J. 2023;2(3):134–148. https://doi.org/10.55940/medphar202351.
17. Rana S, Sharma N, Dutt S, Patil RD. Hepatoprotective Effects of Carissa spinarum Extract on Carbon Tetra Chloride Induced Liver Damage in Zebrafish (Danio rerio). Med Pharm J. 2023;2(4):206–220. https://doi.org/10.55940/medphar202362
18. Naji EM, Naser NH, Hussein SA. In silico study, synthesis, and antineoplastic evaluation of thiazole-based sulfonamide derivatives and their silver complexes with expected carbonic anhydrase inhibitory activity. J Med Life. 2023;16(12):1857–1863. https://doi.org/10.25122/jml-2023-0180.
19. Lee EH, Cho EB, Kim BO, Jung HY, Lee SY, Yoo J, et al. Functional properties of newly-bred 'Summer king'apples. Hortic Sci Technol. 2020;38(3):405-417. https://doi.org/10.7235/HORT.20200039
20. Sayadi V, Karimzadeh G, Rashidi Monfared S, Naghavi, MR. Identification and expression analysis of S-alk (en) yl-L-cysteine sulfoxide lyase isoform genes and determination of allicin contents in Allium species. PLoS One. 2020;15(2):e0228747.
21. Ivanova S, Balkanski S, Atanasov P, Chaneva M, Obreshkova D, Dimitrov V, et al. Antitumor and antioxidant activity of some metal complex compounds. Pharmacia. 2023;70:375–382. https://doi.org/10.3897/pharmacia.70.e105845
22. Trifonova D, Gavrilova A, Dyakova G, Gavrilov G, Yotova M, Nikolov S. Preliminary in vitro study of antioxidant activity and anti-diabetic potential of plant extracts from 4 herbal substances not traditionally used for treatment of diabetes mellitus. Pharmacia. 2021;68(4):755–762. https://doi.org/10.3897/pharmacia.68.e72769
23. Al-Samydai A, Alsamydai MJ, Yousif OR, El Qirem IA. The impact of social media on consumers' health behavior towards choosing herbal cosmetics. J Crit Rev. 2020;7(9):1171-1176. https://doi.org/10.31838/jcr.07.09.214.
24. Sharma S, Chaitanya M, Sharma S, Kumar S, Rustagi S, Singh S, et al. The medicinal plant Berberis aristata and its endophytes for pharmacological applications: Current research and future challenges. J Appl Biol Biotechnol. 2024;12(4):37–46. https://doi.org/10.7324/JABB.2024.167591.
25. Al-Mamoori F, Al-Samydai A, Aburjai T. Medicinal Plants For The Prevention And Management Of Nephrolithiasis: A Review. Int J Sci Tech Res. 2019;8:2700-5.
26. Rambe PS, Putra IB, Yosi A. The effect of roselle leaf (Hibiscus sabdariffa L.) extract gel on wound healing. J Med Life. 2022;15(10):1246–1251. https://doi.org/10.25122/jml-2021-0425.
27. Kumar U, Kumar I, Singh PK, Yadav JS, Dwivedi A, Singh P, et al. Total phenolic content and antioxidant activities in methanol extracts of medicinal herbs from Indo-Gangetic plains of India. J Appl Biol Biotechnol. 2024;12(4):89-99. https://doi.org/10.7324/JABB.2024.172805
28. Kalebar VU, Hoskeri JH, Hiremath SV, Hiremath MB . In vitro antiproliferative effect of aqueous extract of Solanum macranthum fruits on MDA-MB-231 tripple negative breast cancer cell line. J Appl Biol Biotechnol. 2020;8(1):28–32. https://doi.org/10.7324/JABB.2020.80105
29. Yıldırım I, Kutlu T. Anticancer agents: saponin and tannin. Int J Biol Chem. 2015;9(6):332-340. https://doi.org/10.3923/ijbc.2015.332.340
30. Ijarotimi OS, Adesanya IH, Oluwajuyitan TD. Nutritional, antioxidant, angiotensin-converting-enzyme and carbohydrate-hydrolyzing-enzyme inhibitory activities of underutilized leafy vegetable: African wild lettuce (Lactuca taraxacifolia Willd). Clin Phytosci. 2021;7:47. https://doi.org/10.1186/s40816-021-00282-4
31. Al-Halaseh LK, Al-Adaileh S, Mbaideen A, Hajleh MN, Al-Samydai A, Zakaraya ZZ, Dayyih WA. Implication of parabens in cosmetics and cosmeceuticals: Advantages and limitations. J Cosmet Dermatol. 2022;21(8):32653271. https://doi.org/10.1111/jocd.14775
32. Al-Samydai A, Alshaer W, Al-Dujaili EA, Azzam H, Aburjai T. Preparation, characterization, and anticancer effects of capsaicin-loaded nanoliposomes. Nutrients. 2021;13(11):3995. https://doi.org/10.3390/nu13113995.

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