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Evaluation of Anti-tumor Activity of *Ammi majus* seeds Extract on Some Cancer Cell Line

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Abstract

Cancer is one of the major health problems in the world and is one of the important causes of an increase in deaths among children and adults. This study was conducted at the Iraqi Center for Cancer and Medical Genetics Research (ICCMGR) in Baghdad conducted a study between December and March 2023 to evaluate the cytotoxic effect of *Ammi majus* seed extracts on human cancer cell lines (HepG2, Hela and normal line HC). The cancer cell lines were treated with different concentrations (6.25 ,12.5 ,25, 50, 100,200)of the *Ammi majus* seeds extracts (aqueous and ethanolic extracts).The results showed the effect of both seeds extracts (aqueous and alcoholic) on the growth of Hela cell lines increased with increasing the concentration and give highest inhibition rate(3.93 ± 72.69 % and 3.93± 91.87) in respectively ,at a concentration (200 µg/ml,) Whereas the highest inhibition rate on the growth of HepG2 was (3.18 ± 68.86 % and 1.36± 79.31 %) in respectively, at a concentration (200 µg/ml), the highest percentage of inhibition rates on the growth of the HC normal Cell Line were (3.37± 25.25 % and 1.36± 43.52 %) at concentration(200 µg/ml) in respectively . The current study concluded that the aqueous and alcoholic extracts of the *Ammi majus* seeds plant has an effect in the process of inhibiting cancer cell lines making this plant a promising candidate for cancer treatment.

Keyword: Hela cancer cell line; HepG2 cancer cell line, *Ammi majus* seeds extract.

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Introduction

Cancer stands as one of the leading causes of mortality across both children and adults globally, following cardiovascular diseases according to the WHO. It initiates with genetic mutations in DNA, leading to the abnormal proliferation of cells, disregarding growth regulation signals and causing alterations in surrounding tissues [1][2].

Plants have served as medicinal remedies for thousands of years, holding a wealth of bioactive compounds with anti-inflammatory, antimicrobial, and anticancer effects [3]. Various cultures worldwide have extensively utilized herbal medicine, leveraging secondary metabolites like flavonoids, alkaloids, tannins, and steroids found in plants [4].

Ammi species, part of the Umbellifereae family, contain significant bioactive compounds, particularly coumarins and flavonoids, renowned for their biological activities [5]. *Ammi majus* L., known as Bishop's weed or Greater *Ammi*, harbors several active ingredients, including xanthotoxin, bergapten, imperatorin, isoimperatorin, isopimpinellin, and marmesinin [6]. This plant thrives in regions like Iraq, Egypt, the Mediterranean basin, West Africa, Iran, offering a natural source for potential medicinal properties [7]. Over half of today's recognized drugs derive from natural products that can influence cancer cell mechanisms, affecting cell division or DNA replication stages [8]. Furanocoumarins, such as xanthotoxin, imperatorin, and bergapten found in *Ammi majus*, exhibit therapeutic potential against cancer cells by influencing DNA replication and cell division processes.

While chemotherapy remains a prevalent cancer treatment, its non-selective nature leads to the loss of healthy cells alongside cancerous ones, resulting in severe side effects. Addressing this challenge, current research focuses on finding anticancer agents derived from natural sources like *A. majus* seed extracts to treat specific cancer cell lines like Hela and HepG2. This exploration aims to develop treatments that selectively target tumor cells while sparing healthy ones, potentially reducing the severe side effects associated with traditional chemotherapy [9].

Material and Method

The plant material used in this study included *Ammi Majus* seeds purchased from a local herbal market in Diyala Province, Iraq. To authenticate the seeds, were verified by a botanist at the

Iraqi National Herbarium under the Directorate of Seed Analysis and Certification of the Ministry of Agriculture in Abu-Ghraib, Baghdad, Iraq.

Two types of extracts were prepared from the seeds:

1. Aqueous Extract:

The dried seeds were powdered and 50 grams of this powder underwent extraction with 100 ml of distilled water using the Soxhlet apparatus for three hours at 50°C. The resulting extract solution underwent centrifugation (1000 rpm for 15 minutes), followed by collection of the supernatant, which was then evaporated at 50°C using a rotary evaporator [10].

2. Alcoholic Extract:

For this extract, 50 grams of plant powder was subjected to extraction using a thimble in a Soxhlet device with 100ml of 80% ethanol over a period of five hours. The extract obtained from this process was concentrated using a rotary evaporator and subsequently dried in an electric oven at 40°C. The final extract was stored in opaque glass bottles within a refrigerator until use [11].

Cancer Cell Lines

Regarding the cancer cell lines utilized in the study, they were obtained from the Iraqi Center for Cancer and Medical Genetics Research (ICCMGR) in Baghdad , Iraq. Specifically, the Human Liver Cancer Cell Line (HepG2) at step (50) and the human cervical cancer cell line Hela at passage (58).and normal cell line (HC).The study involved several steps to assess the impact of *Ammi majus* extracts on cancer cell lines:

1. Culturing and Maintenance of Cell Line

The Hela and HepG2 cancer cell lines and Normal line HC were grown in RPMI-1640 culture medium supplemented with 10% Fetal Calf Serum (FCS) [11].

The cells were placed in a CO2 incubator that set at 37°C for 24 hours to maintain their viability, eliminate any potential contaminants, and promote regular growth. After incubation, cells were washed with PBS solution and detached using trypsin-versin solution, followed by suspension in fresh culture medium.

2. Activity Assay of *Ammi majus* Extract on Cancer Cell Lines

Initially, a specific volume of RPMI-1640 with Fetal Calf Serum

(FCS) was added to the wells.Various concentrations (6.25, 12.5, 25, 50, 100, 200 µg/ml) of *Ammi majus* extracts were added in triplicate to separate wells, alongsidenegative and positive controls (using DMSO and PBS with serum-free media), respectively [11]. After that the plates were incubated at 37°C until cell adhesion occurred. After removing dead cells and old culture media, crystal violet dyewas added to each well and incubated for 20 minutes. Then the dye was removed, and the wells were washed with PBS to eliminate remnants of the dye. The cells were allowed to dry, and a spectrophotometer was used to measure absorbance at 492 nm. Cell growth inhibition rates were calculated using the formula provided, comparing absorbance readings of control and treated cells for each concentration.

- IC50 (the concentration causing 50% inhibition) for each cancer cell line was determined after 24 hours of exposure [11].

Percentage of inhibition rate (IR) % = (X - Y \ X) x 100

Since:

X = Reading (O.D) of control

Y = Reading (O.D) for each concentration treatment

Analytical statistics

Statistical analysis involved the utilization of ANOVA testing alongside Graph Pad Prism Version 6 for data analysis. The Duncan Multiplex experiment found notable variations between means, indicating significant differences with a probability level of $P \leq 0.05$ [12].

Result

The research was focused on investigating the impact of *Ammi majus* extract on specific human cancer cell lines. Six different concentrations of the extract were administered to distinct cancer cell lines. The findings revealed that both the aqueous and alcoholic extracts displayed inhibitory effects on the growth of the Hela cancer cell line. The most substantial inhibition rates recorded were 3.93 ± 72.69% and 3.93 ± 91.87% at a concentration of 200 µg/ml. These outcomes are comprehensively outlined in Table (1) and visually represented in Figure (1).

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Table 1: Inhibition Percentage in the Hela Cancer Cell Line by the Effect of Different Concentrations for 24 Hours of Exposure at 37° C.

Concentration µg /ml	Inhibition ratio % ± Standard deviation. aquatic extract	Inhibition ratio % ± Standard deviation. alcoholic extract
Control	f 0.01±1.15	j 0.04± 1.17
6.25*	e 0.23±24.89	f 0.23±34.90
12.5*	d 0.33± 31.28	e 0.33±47.73
25*	dc 0.37± 36.21	d 0.37± 61.62
50*	c 0.60± 41.28	c 0.60± 70.46
100*	b 1.12±50.50	b 1.12± 75.08
200*	a 3.93± 72.69	b 3.93± 91.87

Different letters in a column indicate that there are significant differences at the level (*P*<0.05).

• The presence of a mark (*) on the concentration indicates that the two extracts differ significantly from each other at the level (*P*>0.05).

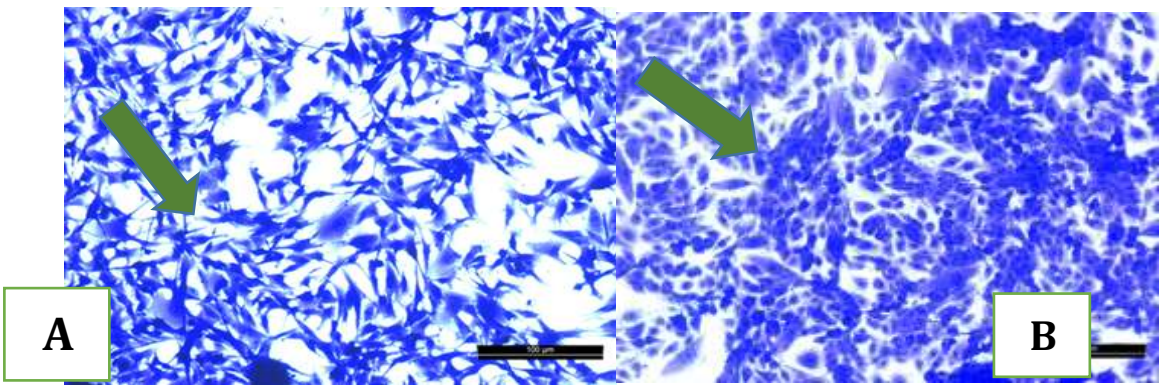

Figure 1: shows a comparison between Hela cell lines treated with alcohol and water extracts at a concentration of 200 µg/ml for 24 hours at 37°C (x100) using Crystal Violet stain. (A) Represents the control group of Hela cancer cell lines, exhibiting dense cell growth when treated with water extract.(B) Shows the Hela cell line treated with alcohol extract at 200 µg/ml, displaying cell death and spaces between cells.

The study findings indicated that both the aqueous and alcoholic extracts had a suppressive effect on the proliferation of the HepG2 Cancer Cell Line. The highest inhibition rates recorded were 3.18 ± 68.86% and 1.36 ± 79.31% at a concentration of 200 µg/ml, respectively. These results are outlined in Table (2) and visualized in Figure (2).

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Table 2: Inhibition Percentage in the HepG2 Cancer Cell Line by the Effect of Different Concentrations of for 24 Hours of Exposure at 37° C.

Concentration µg /ml	Inhibition ratio % ± Standard deviation. aquatic extract	Inhibition ratio % ± Standard deviation. alcoholic extract
Control	f 0. 03± 1.14	j 0.07± 1.15
6.25*	e 0.33±12.53	f 0.58±17.08
12.5*	d 0.48± 17.53	e 0.59±21.35
25*	dc 0.86± 23.82	d 0.63± 32.81
50*	c 1.40± 31.71	c 0.79±52.86
100*	b 1.99±40.15	b 1.20± 67.75
200*	a 3.18± 68.86	a 1.36±79.31

The different letters in one column indicate that there are significant differences at the level (*P*<0.05).

• The presence of a mark (*) on the concentration indicates that the two extracts differ significantly from each other at the level (*P*>0.05).

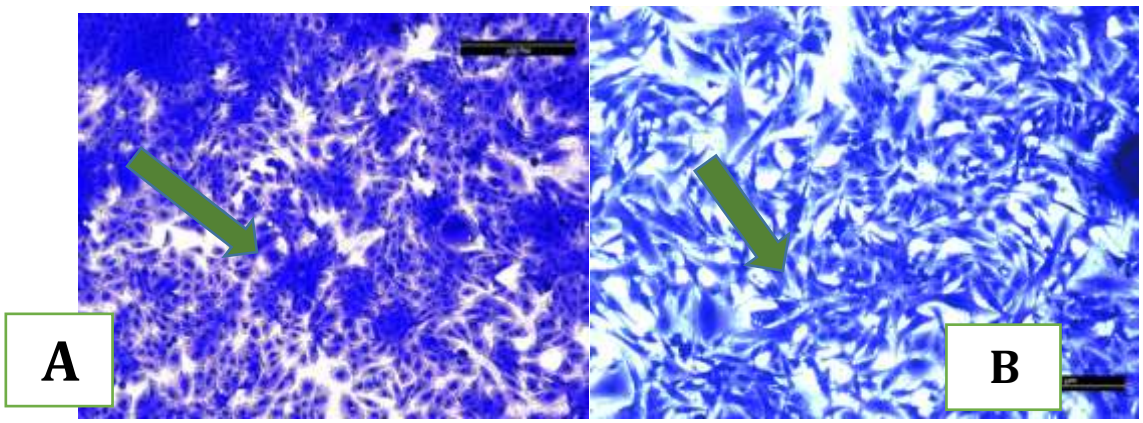

Figure 1: shows comparison between cells of HepG2 line that treated with water extract and alcohol at a concentration of 200 µg/ml for 24 hours exposure at 37° C (x100) by using Crystal Violate stain. (A) HepG2 cancer cell line representing control and showing dense cells. Water extracted (B) HepG2 cell line treated with alcohol extract at a concentration of 200 µg/ml.

The results revealed the aqueous and alcoholic extracts had an inhibitory effect on the growth of the HC normal Cell Line and the highest percentage of inhibition rates were (3.37± 25.25 % and 1.36± 43.52 %) at concentration(200 µg/ml) in respectively ,as shown in Table (3) and Figure (3).

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Table 3 :Inhibition percentage in the HC normal Cell Line by the Effect of Different Concentrations for 24Hours Exposure at 37° C

Concentration µg /ml	Inhibition ratio % ± Standard deviation. aquatic extract	Inhibition ratio % ± Standard deviation. alcoholic extract
Control	e 0.04±1.15	e 0.08± 1.15
6.25*	d 0.34± 6.29	d 0.15±6.98
12.5*	d 0.25±9.60	c 0.36± 11.48
25*	c 0.60± 10.55	b 0.56±14.17
50*	c 0.18±13.78	b 0.62±18.08
100*	b 0.78± 18.61	b 1.31±23.32
200*	a 3.37± 25.25	a 1.36± 43.52

The different letters in one column indicate that there are significant differences at the level (*P*<0.05).

• The presence of a mark (*) on the concentration indicates that the two extracts differ significantly from each other at the level *(P*>0.05

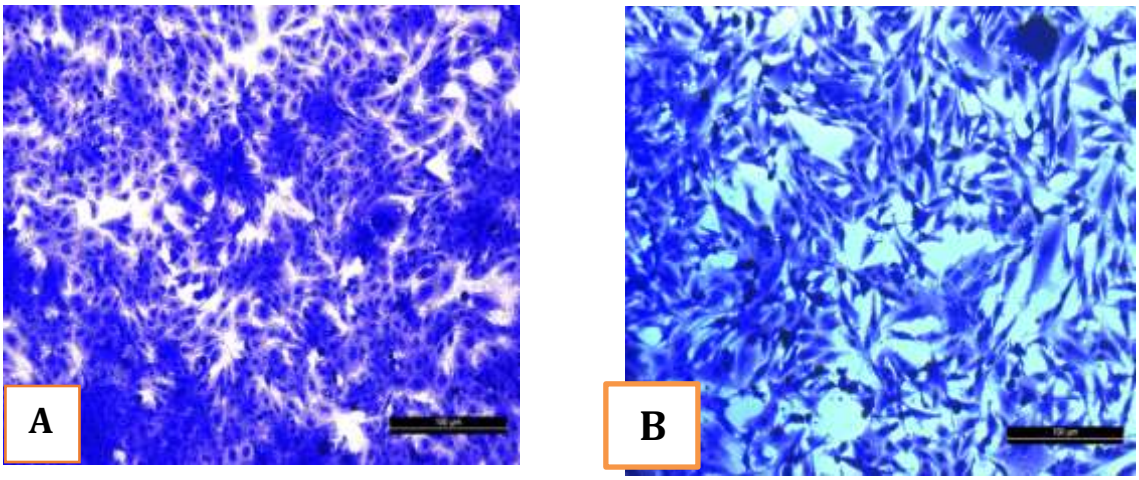

Figure 3: comparison between cells of HC line that treated water and alcohol extract at a concentration of 200 µg/ml for 24 hours exposure at 37° C (x100) by using Crystal Violate stain. (A) HC cancer cell line representing control and showing dense cells.water extraction (B) HC cell line treated with alcohol extract at a concentration of 200 µg/ml and showing dead cells and voids between cells.

Discussion

The research findings reinforced previous studies highlighting the anticancer potential of plant extracts, underscoring that their efficacy hinges on the extract type, concentration, and the sensitivity of cancer cells [13]. Both aqueous and alcoholic extracts displayed inhibitory effects

across all concentrations on the growth of Hela and HepG2 cancer cell lines and HC nomal line were in detailed in Tables (1, 2 and 3).

Specific compounds within *Ammi majus*, such as xanthotoxin, have demonstrated promising anticancer effects with favorable compatibility with normal human cells, necessitating further investigations to optimize their efficacy, toxicity, solubility, and pharmacokinetics [14].

Furanocoumarin derivatives found in the plant contribute to its defense mechanism by bolstering Systemic Acquired Resistance (SAR) against external threats, aiding in suppressing microbial growth and alleviating inflammation. Recent evidence highlights their potential in cancer treatment [8].

Studies have emphasized the inhibitory effects of the ethanolic extract of *Ammi majus* on cancer cell growth, particularly on MCF7 and Hela cells. Coumarin compounds, notably psoralens within this plant, exhibit cytotoxicity on cell lineages and induce apoptosis, contributing to their potential anticancer role by inhibiting cytochrome P450 activity [5, 15].

Bioactive compounds derived from food and plants play pivotal roles in preventing and treating diseases like HCC [16], offering advantages such as easy accessibility and economic value. Their efficacy in scavenging free radicals, stimulating apoptosis, and inhibiting cancer cell survival is a driving force behind their increasing demand [17].

The quest for new drug candidates involves diverse approaches, aiming either to discover cytotoxic agents or enhance the selectivity and activity of existing anticancer drugs [18].

Studies exploring natural plant sources in Iraq, such as *Ammi majus*, reveal their historical use and recent investigations highlighting their anticancer potential. Extracts from *Ammi visnaga*, for instance, exhibited anticancer activity against hepatic cancer, while coumarin types isolated from *Ammi majus* displayed high activity on various cell lines [19]. The cytotoxicity of coumarin types were isolated from the aerial parts of the wild medicinal plant *Ammi majus*, depending on their structure features had high activity on different cell lines (HCT116 cell line) (20).

In conclusion, the study demonstrated that high concentrations of *Ammi majus L*. seed extracts, both aqueous and alcoholic, can inhibit the growth of Hela and HepG2 cancer cell lines. These

promising results prompt further research into the anticancer potential of *A. majus*, albeit necessitating more extensive studies for definitive proof.

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