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تقييم الفعالية البيولوجية للمستخلصات الخام لبذور *Ammi majus (L.)* داخل وخارج الجسم الحي

رسالة مقدمة إلى مجلس كلية العلوم- جامعة ديالى

وهي جزء من متطلبات نيل شهادة الماجستير في علوم الحياة

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إشراف

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Summary

The *Ammi majus*, plant is frequently utilized for addressing various health issues. This study aimed to investigate the biological activities of *Ammi majus* seeds extracts, with a specific focus on its antibacterial and anti-inflammatory properties in mice, assessed through a histopathological study of kidney tissue. Furthermore, the study aimed to evaluate the efficacy of its aqueous and alcoholic extracts in treating specific cancerous human cell lines, namely HepG2 and Hela cell line.

The present study was conducted at the Biology Department, College of Science, Diyala University, and the Iraqi Center for Cancer and Medical Genetics Research (ICCMGR) in Baghdad, spanning from October 2022 to March 2023. The antibacterial activity was assessed using the agar well diffusion method, involving five bacterial species isolated from patients with chronic urinary tract infections, namely (*Escherichia coli, Staphylococcus aureus, Acinetobacter boumannii, Pseudomonas aeruginosa*, and *Proteus spp*). Furthermore, the study involved the determination of the anti-inflammatory effects of *Ammi majus* seeds extracts in mice. This was achieved through microscopic examination of stained tissue sections of kidneys obtained from deceased mice after four days.

A cytotoxic test was conducted to assess the impact of both aqueous and alcoholic seeds extracts of *Ammi majus* on the growth of cancerous tumors. Cancer cell lines, including Hela, HepG2, and the normal line HC, were utilized to evaluate the extract's effect on the proliferative capacity of cancer cells. The cancer cell lines were exposed to six concentrations (6.25, 12.5, 25, 50, 100, and 200 µg/ml), and the extracts were sterilized using a 0.22µm syringe filter unit under sterile conditions.

The results of the antibacterial activity of the crude extract of *A. majus* seeds showed that the highest inhibition zone was observed in *E. coli* (25.7 mm), *S. aureus* (23.3 mm), *A. baumannii* (17.7 mm), *P. aeruginosa* (16.7 mm), and *Proteus spp*. (15.4 mm).

In the mice studied, the results of the total WBC count and biochemical analysis revealed notable findings. Treating mice with pathogenic bacteria (*E. coli*) in the positive control group led to a increased WBC count (8.8±0.15 cells/cu.mm) compared to other treatments. However, mice treated with plant extracts at concentrations of 10% and 25% exhibited an decrease in WBC count (4.5±0.03 and 3.7±0.07 cells/cu.mm), respectively.Furthermore, the injection of mice with pathogenic bacteria (*E. coli*) significantly elevated serum urea, ESR, and CRP levels (84±11.3 and 8, and 4.7 unit/l) respectively, compared with a negative control group (32.7±3.6, 4, and 3.6 unit/l) respectively. Notably, the treatment of mice with the alcoholic extract of *A. majus* plant at a concentration of 25% resulted in reduced urea values, ESR, and CRP (38.7±3.9, 4, 4.3) unit/l, respectively.

The results of the microscopic examination of stained tissue sections of the kidneys from Group 2, which were infected with *E. coli*, showed necrotic lesions in renal parenchyma tubules, congestion with inflammatory exudate (edema), and degeneration changes. Additionally, there were congested blood vessels with mononuclear cell infiltration around and between glomeruli, along with acute cellular degeneration of renal tubules.

Contrastingly, the results of Group 3, which were infected with *E. coli* and treated with antibiotics, showed numerous congested areas with degenerative changes in renal tubules. Some areas exhibited sloughing in renal tubules with necrosis in the epithelium and inflammatory exudate, accompanied by degenerative variations in renal tubules.

By examining the outcomes of tissue sections from Groups 4, 5, and 6, which were infected with *E. coli* and subsequently treated with crude aqueous extract derived from the seeds of *Ammi majus* at three concentrations (5%, 10%, and 25%) respectively, it was observed that the extract of *Ammi majus* at a concentration of 25% (Group 6) was successful in treating the infection and provided the best results. The kidney parenchyma showed normal tissue with no clear lesions.

The cancer cell lines were treated with different concentrations (6.25, 12.5, 25, 50, 100, 200) of *Ammi majus* seeds extracts (aqueous and ethanolic extracts). The results showed that the effect of both seed extracts (aqueous and alcoholic) on the growth of Hela cell lines increased with increasing concentration, reaching the highest inhibition rate (3.93 ± 72.69% and 3.93± 91.87%) respectively, at a concentration of 200 µg/ml. Similarly, the highest inhibition rate for the seed extracts (aqueous and alcoholic) on the growth of HepG2 was (3.18 ± 68.86% and 1.36 ± 79.31%) respectively, at a concentration of 200 µg/ml. The results also revealed that the aqueous and alcoholic extracts had an inhibitory effect on the growth of the HC normal Cell Line, with the highest percentage of inhibition rates being (3.37± 25.25% and 1.36± 43.52%) at a concentration of 200 µg/ml.

Finally, the current study concluded that the alcoholic and aqueous extracts of *A. majus* seeds using as antibacterial in vivo and in vitro and have an inhibitory effect on the growth of cancer cell lines, making this plant a promising candidate for cancer treatment.

Chapter One
Introduction

1-Introduction

Plants serve as a valuable reservoir of secondary metabolites essential for various applications such as pharmaceuticals, flavors, fragrances, colors, bio-pesticides, and food additives. Undoubtedly, they represent a natural gift to humanity, contributing to the promotion of health (Al-Snafi, 2013). Over 80% of the world population relies on plant-based medication for basic healthcare needs, a system that has evolved through dynamic interactions between people and their environment (Laird, 2003). Presently, plants and their derivatives play crucial roles in traditional, botanical, and pharmaceutical medicine (Magdum and Kumar, 2013). Recent research endeavors aim to explore novel treatment methods using natural sources like plants, which harbor numerous bioactive compounds (Guarrera, 2005). The therapeutic properties attributed to these plants stem from the presence of secondary metabolites such as flavonoids, alkaloids, tannins, and steroids. Over generations, various cultures worldwide have accumulated extensive knowledge about herbal medicine (Al-Snafi, 2015).

The *Ammi* species, belonging to the Umbelliferae family, contains bioactive compounds, primarily coumarins and flavonoids, which exhibit significant biological activities. *Ammi majus L*. (Apiaceae) is known by various names such as Bishop's weed and Greater Ammi in English, and Khella shaitani and Khella baria in Arabic. The seeds of *Ammi majus* contain various active ingredients, including xanthotoxin, bergapten, imperatorin, isoimperatorin, isopimpinellin, and marmesinin (Walters *et al*., 1996; Al-Snafi, 2013).

In Iraq, *Ammi majus (A. majus)* is commonly found in fields, gardens, and drains, often considered weeds in agriculture. It is collected in places like Kut, Baghdad, and Hawaija. However, *Ammi visnaga* is found in northern Iraq, especially in Erbil, Mosul, Baghdad, Sulaymaniyah, and Kirkuk. It is also found along the Mediterranean Sea, in

West Africa, in parts of Iran and the Kohaj Mountains, in North Africa, in Europe, in the eastern Mediterranean region, in Southwest Asia, in North America, in Argentina, in Chile, in Mexico, and on the Atlantic Island (Al-Snafi, 2013).

The chemical compounds in *Ammi majus* include furanocoumarins, such as bergapten (heraclin, majudin, 5-methoxypsoralen, marmesin, isoperetorin, heraclenin, and isopimelin), xanthotoxins (methoxselene, 8-methoxypsorane, amoidin), and imperatorin (ammidin) (Joy *et al*., 1998; Al-Snafi, 2015). *Ammi majus* seeds are particularly rich in the group of chemicals known as furocoumarins. Twelve specific furocoumarins account for 72.8% of the total seed constituents identified in *A. majus* L. seeds (Pokrovskii *et al*., 2009).

Cancer is a major health issue worldwide and has been ranked as one of the leading causes of death in both children and adults. According to the World Health Organization (WHO) (Krupa-Kotara and Dakowska, 2021), cancer is the second leading cause of death worldwide after cardiovascular diseases (Sunget *et al*., 2021; Siegel *et al*., 2022).

Furanocoumarin derivatives are essential components of the plant *Ammi majus* defense mechanism, exerting systemic acquired resistance (SAR) to combat external threats. By inhibiting or attenuating microorganisms in infected parts, these factors, along with SAR, contribute to the reduction of inflammation in the human body. Recent evidence suggests their potential in cancer treatment (Acharya, 2022).

The seeds of *A. majus* contain important active compounds, particularly coumarin and flavonoids, that are recognized for their anti-inflammatory activities. These compounds suggest potential benefits in the treatment of diseases with asthma-like symptoms (Selim and Ouf, 2012).

Bacterial infections pose a significant challenge in medicine, with a major contributing factor being that a large percentage of these infections are endogenous, meaning that the causative agents originate from the human bacterial flora (Kolář, 2022). Antibiotics have played a crucial role in preventing disease-causing microorganisms, significantly improving people's health-related quality of life. However, the effectiveness of antibiotics has been compromised in recent decades. This is attributed not only to the potential for toxic reactions caused by many widely used antibiotics but also to the emergence of drug-resistant microbes. This renders some antibiotics less effective against certain infections (Bhalodia and Shukla, 2011).

Aims of the Study

The focus of this study is on *Ammi majus* seeds, inspired by their traditional use in folk medicine to treat various disorders. The study aims to evaluate several biological effects of *A. majus* seed extracts through the following steps:

1. Preparation of aqueous and ethanolic seed extracts of *A. majus* using maceration and Soxhlet apparatus respectively.
2. Detection of the antibacterial activity of crude extracts of *A. majus* on various bacterial species (both gram-positive and gram-negative) using the well diffusion in agar method.
3. Detection and evaluation of the treatment effect of the crude extract of *A. majus* seeds, comparing it with antibiotics.
4. Determination of the cytotoxicity and anti-tumor effect of both aqueous and ethanolic crude extracts of *A. majus* seeds..