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Phytochemical Screening and Bioactivity Study of *Ammi Visnaga*

By:

Ghufran Saadi

Hawaraa Kadhim

Ameer Kadhim

Supervised By:

Dr. Aseel Mohammed Omran

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Supervisor Certification

I certify that this project was prepared by the fifth year student under my/our supervision at College of Pharmacy, Babylon University in Partial fulfillment of graduation requirements for the Bachelor Degree in Pharmacy.

Signature:

Name of the supervisor: Dr. Aseel Mohammed Omran

Date:

الاهداء

حينما يكون الجهد مميزاً والعطاء فاعلاً تسمو النفوس إلى مرافئ الإبداع

وترتقي منار التميز عندها يصبح للشكر معنى وللثناء فائدة

وبعبير الحروف والكلمات وشذى احلى العبارات أود أن أوجه شكري

إلى داعمي الأول وسندي في هذه الحياة ...الله عز وجل

إلى أملي الغائب الحاضر ...عزيز الزهراء (ع)

إلى أجمل الوان الامان الي استظل بظل شيبه الذي يضاهي الغيم بياضا... أبي

إلى من الجنة تحت قدميها والدعاء لنا مستجاب من فوق يديها ...أمي

إلى من فسر لي الكتاب وذلل لي الصعاب وصرت بفضلهِ من ذوي الألباب...دكتورتي الفاضلة

د.اسيل محمد عمران

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

{ وَمَا تَوْفِيقِي إِلَّا بِاللَّهِ }

صدق الله العلي العظيم

[سورة هود من الآية:88]

Acknowledgement

I would like to thank my first and only support in this life, I own all my success to ALLAH, for now and ever I will not stop fighting while ALLAH by my side.

I would like to thank Babylon University, Babil –Iraq, forits support in the present work

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I would like to thank my supervisor Dr. Aseel Mohammed Omran for her support and Motivation .....

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Introduction

Ammi visnaga is an annular plant grows to approximately 120 cm height and belongs to Family Apiaceae (fig. 1.1). The plant is endogenous to Egypt and other regions in the Middle East. It is known as Khella, has a slight aromatic odor and a very bitter taste. [5]

Fig. 1.1 Ammi Visnaga [4]

In Egypt, the plant is widely distributed in the Delta region, and surrounds the Nile River, particularly in Assiut and Minia governorates. It is also widely cultivated by many people and companies aiming to use its extracts or active components in the pharmaceutical industry. [6]

The chemical constituents of A. visnaga include various chemical constituents which are **γ-pyrones, coumarins flavonoids, and essential oils**. The quality and

quantity of these secondary metabolites depend on the part of the plant analysed, as well as the growing conditions and the addition of any bioregulators. [3]

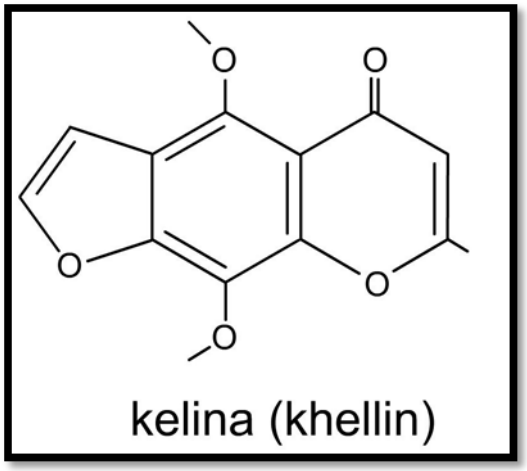


Fig. 1.2 Chemical structure of khellin

The major constituents of A. visnaga are **γ-pyrones** (furanochromone derivatives) (fig.1.3), which are up to 4%. Among the γ-pyrones, khellin (0.3-1.2%) and visnagin (0.05-0.30%) are the major ones. Khellinol, ammiol, visammiol, khellol, khellinin, khellinone, visnaginone are other important γ-pyrones. Coumarins (0.2-0.5%) is another important group of major constituents, the main one being the pyranocoumarins/visnagans (0.2-0.5%) comprising mainly of visnadin, samidin and dihydrosamidin

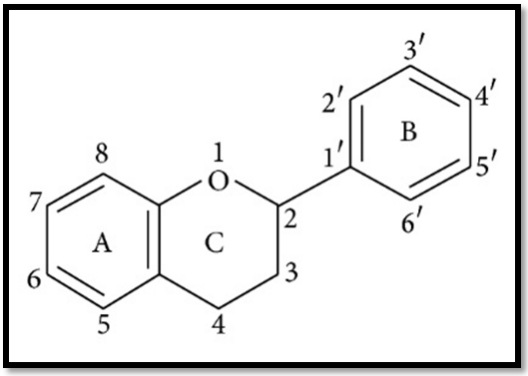


Fig. 1.3 Chemical structures of γ-pyrones.

Two flavonols (quercetin and kaempferol) were identified in A. visnaga growing in Iraq (fig. 1.4). Eleven flavonols were isolated from the aerial parts of A. Visnaga. There were four aglycones, four monoglycosides, two diglycosides and one triglycoside. Among the aglycones flavonoids, one was hydroxylated (quercetin) and three methoxylated (rhamnetin, isorhamnetin and rhamnazin). [1]

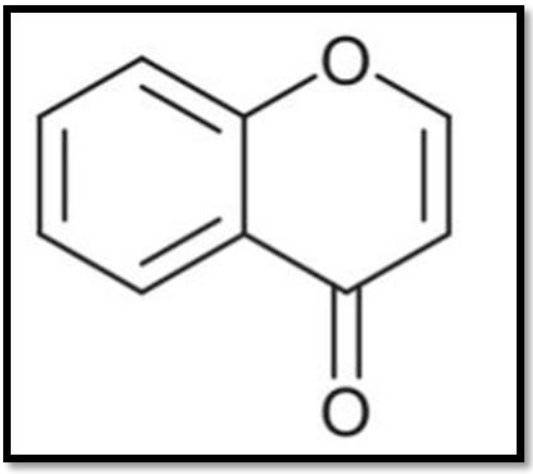


Fig. 1.4 Chemical structure of flavonols.

The main compound of **essential oils** (fig. 1.5) of A. visnaga reported in the essential oils of A. Visnaga from Marocoo were amyl isobutyrate (16%), linalool (22.7%), methyl-2-isoamyl butyrate (27.7%) and amyl valerate (~10%). Forty-one constituents were identified in the essential oils of A. visnaga fruits, collected from Ichkeul and Djebba, the North of Tunisia. The essential oils from both samples were having high percentages of non-terpene esters (43.3 to 49.1%) and oxygenated monoterpenes (38.5 to 39.1%). [2]

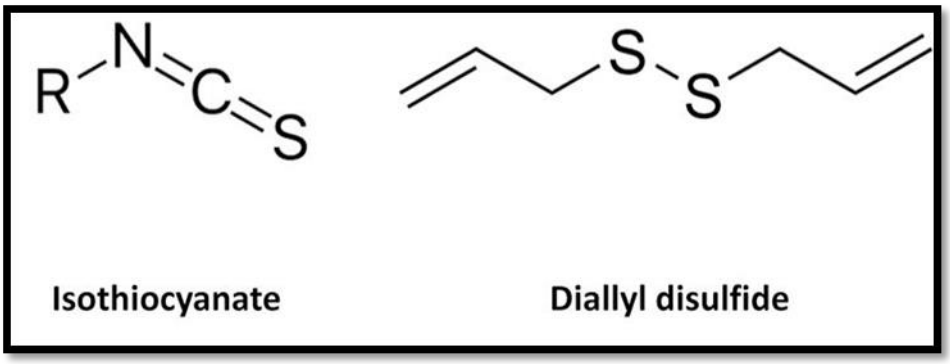


Fig. 1.5 chemical structures of essential oils

They have wide medical uses, they may have cardiovascular effects which attributed to calcium channel blocking actions. It can inhibit vascular smooth muscle contraction and may dilate peripheral and coronary vessels and increase coronary circulation. [7]

The khellin constituent also acts as a vasodilator and has bronchodilatory activity. There is some preliminary evidence that khellin might also increase HDL-cholesterol levels without affecting total cholesterol or triglyceride concentrations.

A khella extract seems to have some antimicrobial activity. This might be attributable to both the khellin and visnagin constituents, which both seem to have antifungal, antibacterial, and antiviral activity. Some studies show ability to khella in psoriasis. The khellin constituent is structurally similar to the psoralen nucleus and might be useful as a photosensitizer in patients with psoriasis.[7]

Orally, khella is used as antispasmodic for colic and abdominal cramps, kidney stones, menstrual pain, and premenstrual syndrome. [7]

Khella is also used for respiratory conditions including asthma, bronchitis, cough, and whooping cough. It is also used for cardiovascular disorders including hypertension, cardiac arrhythmias, congestive heart failure, angina, atherosclerosis, and hypercholesterolemia. It is also used for liver and gall bladder disorders,

diabetes, and as a diuretic. Topically, khella is used on the skin for vitiligo, psoriasis, patchy hair loss (alopecia areata), wound healing, inflammation conditions, and poisonous bites. [7]

Chapter One: Review of Literature

1. Medical Uses of Ammi Visnaga

There is wide range of medical uses of khella (A. Visnaga), it could relieve a number of ailments, such as the acute pain caused by a reduction in the flow of blood to the heart. In addition, A. visnaga is considered anti-asthmatic, diuretic, vasodilator and an effective muscle relaxant. And also it has been used to alleviate the severe pain of kidney stones. [8]

The seeds contain khellin, the chemical constituent considered as a selective coronary vasodilator and also used in the treatment of asthma. Further, both the extract and constituents of A. visnaga have antispasmodic action and also dilate bronchial, urinary and blood vessels without affecting blood pressure. Essential oil of A. visnaga is well-known for its efficacy against coronary diseases and bronchial asthma. [8]

1.1 Antimicrobial Effect

Khella (A. Visnaga) is considered to have antimicrobial activities. Generally the antimicrobial activities were associated with khellin and visnagin. Both these constituents were considered to have antifungal, antibacterial, and antiviral activities. Because of the antimicrobial activities, A. visnaga could also be used for curing psoriasis, probably because of the structural similarity between khellin and psoralen. A. visnaga has photo-sensitizing ability and it was considered useful as a photo-sensitizer in patients with psoriasis. The following examples do authenticate the antimicrobial potential of A. visnaga. Its fruit extract in 95% ethanol exhibited antibacterial activity, inhibiting the growth of Mycobacterium tuberculosis H37RVTMC 102 even in a very low concentration (dilution of 1:40).[10]

Similarly, 50% acetone, 50% aqueous or 95% ethanol extract of A. visnaga inhibited fungal growth (Neurospora crassa) In- vitro. Again, the aqueous extract of its fruits (in a concentration range of 210 mg/ml inhibited the growth and aflatoxin production by Aspergillus flavus. Ethanolic and aqueous extract of the A. visnaga were tested against eight pathogenic microorganisms. The most active extract against Gram-positive bacteria was ethanol extract with MIC value (5 mg/ml) against Enterococcus faecalis. Though, a high concentration of extract was required to cause inhibition in yeast.[9]

When the essential oil of A. visnaga was tested against E. coli and different other bacteria, it showed the best antibacterial activity against E. coli, Staphylococcus aureus and Pseudomonas aruginosa. Similarly the aqueous and hydro-alcoholic extract of seed and stem of A. visnaga had a remarkable antibacterial activity against S. mutans, S. salivarius and S. Sangguis. [11]

1.2 Cytotoxic Effect

Many studies found that khellin has cytotoxicity against four human tumour cell lines: HT-29 (colorectal cancer), MCF-7 (breast cancer), HEp-2 (larynx cancer), and MKN-45 (gastric cancer). However, the results were not promising, and the substance did not show significant cytotoxic activity at the tested concentrations against the four cell lines. While other studies has been showed khellin has mild to moderate activity when tested against the hepatocarcinoma cell line (HepG2). [12]

An ethanolic extract of A. visnaga also showed inhibitory effects on both Hela (cervical cancer) and MCF7 cell lines. The cytotoxic activity of isolated khellin and visnagin against four human cell lines, Hela (cervical carcinoma), Hep-G2 (liver carcinoama), HCT 116 (colon carcinoma), and MCF7 (breast carcinoma),

was further investigated; the results revealed good cytotoxic activity of both γ-pyrones against the Hep-G2 cell line. [6]

1.3 Antispasmodic Effect

visnaga is known to support body to combat spasms in smooth muscles and dilate blood vessels and therefore, its antispasmodic properties are very much valuable to treat asthma attacks. Now it is known that khellin and visnagin mitigate spasms in the bronchial passages. [13]

Visnadin also caused nonspecific inhibition of vascular smooth muscles and selectively inhibited the contractile response in the rat aortic ring and portal vein segment. [14]

visnaga could induce relaxation of smooth muscles, including that of the ureter and coronary arteries, in a variety of animal species. A very slight amount of A. visnaga's seeds could relieve the throbbing through its antispasmodic effects on the urinary tract muscles. For similar reasons, a number of asthma medications were formulated using A. visnaga in 1950s. [13]

1.4 Antioxidant Activity

Very few studies have examined the antioxidant properties of A. visnaga. The free radical scavenging activity of the butanol extracts of the aerial parts of A. visnaga has been investigated, showing equivalent antioxidant activity, i.e., an IC50 equals to 8.77 ± 0.2 µg/mL, to the standard antioxidant rutin (IC50 = 3.01 ± 0.2 µg/mL). [6]

Another study examined the antioxidant activity of essential oils isolated from the umbels of A. visnaga; however, the results showed only very weak activity. [15]

1.5 Antimutagenic Effect

In a study aiming to evaluate the antimutagenicity spectrum of A. visnaga, khellin showed inhibition to mutagenicity of promutagens benzo[a]pyrene, 2-aminofluorene, and 2- aminoanthracene in Salmonella typhimurium T98, while visnagin showed higher toxic activity. Meanwhile, the total extract from A. visnaga fruit showed higher inhibition potency than khellin alone against 2-aminoanthracene, 1-nitropyrene, and daunomycin. This was attributed to the presence of additional inhibitors such as coumarins, or to the synergistic effects with the accompanying compounds. [6]

1.6 Immunostimulatory Activity

A. visnaga total and protein extracts were found to have immunostimulatory effects. Extracts were tested using an MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) assay on splenocytes with or without stimulation by concanavalin-A (Con-A), a mitogenic agent used as a positive control. This could explain the traditional use of such a plant. [16]

1.7 Cardiovascular Effect

The seeds of khella (A. Visnaga) are known to relieve the severe pain caused by a reduction in the blood flow to the heart. These properties of A. visnaga are attributed to its γ-pyrone constituents. The three constituents, visnadin, visnagin, and khellin, all are considered to have cardiovascular effects mainly because of their calcium channel blocking activities. Visnadin was found the most active when used in In-vitro experiments. It inhibited vascular smooth muscle contraction and caused the dilatation of peripheral and coronary vessels and an increase in coronary circulation. [8]

Visnagin, also exhibited peripheral and coronary vasodilator activities and has

been used for the treatment of angina pectoris as it caused non-specific inhibition of vascular smooth muscle contractility. Further, visnagin has negative inotropic and chronotropic effects and helps in reduction of peripheral vascular resistance. Khellin and visnagin both are capable of inhibiting the spasms, indicating an involvement of a calcium channel blocking mode of action. Further khellin increased HDL-cholesterol in normo-lipidaemic subjects. Therefore, khellin also acts as a vasodilator and has bronchodilatory and spasmolytic activity. [8]

1.8 Anti-Inflammatory effect

The anti-inflammatory effects of A. visnaga have been investigated, and it was shown that, depending on its visnagin content, it caused a decrease in mRNA expression and the release of TNFα, IL-1β, and IFNγ. In addition, visnagin reduced LPS-induced IL-6 and MCP-1 mRNA level, thus suggesting that the anti-inflammatory effect of visnagin may be due to the inhibition of transcription factors such as AP-1 and NF-κB. [6]

Moreover, Kwon et al. suggested that visnagin had a neuroprotective effect in terms of suppressing kainic acid-induced pathogenesis in the brain, and that these neuroprotective effects are associated with its anti-inflammatory effects. [17]

1.9 Treatment of Kidney Disease

Ammi visnaga has been widely used in treatment of kidney disease, its use had spread to the extent that it was regarded as the most recommended species for the treatment of urinary tract infections. [6]

Several studies have focused on the diuretic activity of A. visnaga; it has been shown to be effective in the treatment of nephrolithiasis and uremia. Its use in the treatment of kidney disorders is commonly coupled with khellin and visnagin, i.e.,

the major γ-pyrones of A. visnaga. They have been shown to protect the renal epithelial cell damage from oxalate and calcium oxalate monohydrate crystals, and to prevent the oxalate formation that is associated with hyperoxaluria by increasing the urinary pH and citrate concentration, along with a decrease of urinary oxalates. [18]

Ammi visnaga also effective in treatment of urolithiasis which is clinical condition referred to as kidney stone disease. These stones are usually composed mainly of calcium oxalate. Several factors are involved in the formation of kidney stones such as dehydration, consumption of certain foods containing high amount of calcium, oxalate or uric acid and some infectious diseases. [10]

It was found that daily oral treatment with A. visnaga (500 mg/kg) could inhibit the formation of kidney stones by lowering the deposition of calculi in kidney. The prophylactic effect of A. visnaga was attributed to its diuretic activity. Another study evaluated the effect of A. visnaga and its two major constituents (khellin and visnagin) could play a strong role in the prevention of stone formation due to hyperoxaluria. [10]

Many studies foumd that oral administeretion of A. visnaga extract (125, 250 or 500 mg/kg) for 14 days. Rationally, a good correlation was obtained between the incidence of crystal deposition and the increase in urine pH. This study also demonstrated that A. visnaga could be used as a possible therapeutic approach for the prevention of kidney stones due to hyperoxaluria. [10]

In another similar research study, the effect of aqueous seeds extracts and its two constituents khellin and visnagin was observed on the crystal deposition in stone forming rates. But both the constituents did not affect urinary citrate or oxalate excretion, unlike aqueous seed extract, signifying a mechanism of action different

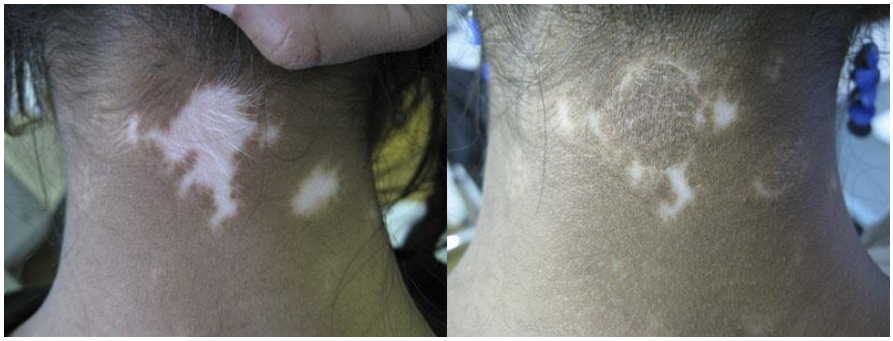
from the aqueous seed extract. The inhibitory effect of A. visnaga extract (aqueous extract of whole plant and its seeds) was studied on the oxalocalcic crystallization in human urine. Even this study revealed the efficacy of extracts of the A. visnaga seeds in inhibiting the crystallization of calcium oxalate. Further, it was found that the extracts reduced oxalate calcium crystallization and specially monohydrate oxalate calcium. [19]

1.10 Treatment of Vitiligo

Vitiligo, also called leukoderma or white skin, is a skin disease, wherein there is a steady loss of the melanin pigment from the skin layers often with a progressive course causing destruction of melanocytes. As mentioned, topically A. visnaga is applied for curing vitiligo and psoriasis. The reason is that A. visnaga possesses phototherapeutic properties similar to those of the psoralens, however with significantly lesser phototoxic and DNA mutation effects. [21]

Fig.1.6 Ammi visnaga cream

Since 1982, khellin has been shown to be effective in both oral and topical photochemotherapy for the treatment of vitiligo. Many studies suggest that the treatment with a gel formulation of khellin based upon a water/2-propanolpropylene glycol (khellin-WPG) system combined with ultraviolet A (UVA) significantly improved the clinical outcome of patients with vitiligo by facilitating the availability of the drug in the skin. [6]



A **B**

Fig. 1.7 Uses of ammi visnaga for treatment of vitiligo (A) before treatment and (B) After treatment.

1.11 Treatment Hair Loss

The topical application of A. visnaga for hair loss has been studied. A lotion for hair scalp composed of visnadin and other constituents led to an increase in arterial and arteriolar sphygmic activity in the subpapillary plexus, leading to an improvement in local microcirculatory flow. [6]

1.12 Herbicidal Activity

A study done in Argentina found that the dichloromethane extract of A. visnaga had a significant herbicidal effect. Phytotoxicity fractionation was done, and the fraction that contained khellin and visnagin was found to be responsible for its significant herbicidal activity. [21]

2. Dose of Ammi Visnaga

The daily recommended dose from A. visnaga fruit ranges from 0.05 to 0.15 g. For other dosage forms, the dose must be mentioned in the insert leaflet, as directed by the physician. [6]

3. Adverse Effects of Ammin Visnga

Traditionally, A. visnaga is used an emmenagogue and its fruits or extracts should be avoided during pregnancy. Intake of A. visnaga is not recommended at all along with blood thinners such as coumadin, anti-hypertensive drugs like calcium channel blockers or other drugs that lower blood pressure. During treatment with A. visnaga and its constituents, the exposure to sun or other sources of ultraviolet light should be avoided, in order to minimize photosensitivity. Overdose or longer use of the A. visnaga can lead to queasiness, dizziness, loss of appetite, headache, sleep disorders. Similarly, side effects like pseudoallergic reactions, reversible cholestatic jaundice and elevated activities of liver transaminases and γglutamyltransferase have been observed with the use of A. visnaga or its constituents. [8]

Fig. 8 Adverse effect and contraindication of Ammi Visnaga

Chapter Two: Practical Section

2.1 Materials and Methods

Preparation of Implant Extracts

Preparation of Aqueous Extracts

Aqueous extracts has been prepared by 10 g from each plant (air-dried powder) then taken and placed in conical flask (500 ml), and then adding of distilled water, and boiled on slow heat for 2 hours, then filtered through 8 layers of muslin cloth and centrifuged at 5000 RPM for 10 min. The supernatant was collected. Then the extract was concentrated in an oven at 45^{o}c until dryness. Dried extracts were stored at 4^{o}c for further use.

Preparation of Alcoholic Extracts

10 gm of the powdered plant was soaked in the conical flask (250 ml) containing 200 ml of ethanol, plugged with cotton, and then put in a horizontal shaker at 140-220 RPM for 24 h. Then it was filtered through 8 layers of muslin cloth and centrifuged at 5000 RPM for 10 min, the supernatant was collected. The extract was concentrated in an oven at 45^{o}c until dryness. Dried extracts was stored at 4^{o}c for further use.

Qualitative tests of phytochemicals:-

A. Tests for Phenols

1. Tests for Lead Acetate

Lead acetate test .The extract (50 mg) was dissolved in 5 mL of distilled water. To this, 3ml of 10% lead acetate were added. A bulky white precipitate indicated the presence of phenol compounds (Tamilselvi *et al*., 2012).

2. Tests for Tannin

Ferric chloride**:** A portion of extract was dissolved in water, then filtrate it, Ferric chloride (FeCl3) (1%) was added. The appearance of black color indicates the presence of tannins (Anyasor *et al*., 2010).

3. Gelatin Test

1g of gelatin was dissolved in 10 ml of distilled water and 1 g of sodium chloride dissolved in 10 ml of distilled water and then we add aqueous extracts and alcoholic extracts.

4. Saponin Test

We add 5 ml of aqueous extracts and alcoholic extracts in tube and then we make rapid shaking.

B. Tests for Alkaloids

1. Mayer's Test

This reagent prepared by dissolving 13.5 gm from Mercuric chloride and 5 gm from Kl in 1000 ml distilled water, add 1-2 ml from reagent to 5 ml from extract. A white or creamy precipitate was indicated the test as positive (Harborne, 1984).

2. Hagere's Test

Hager’s reagent is saturated solution of picric acid (C6H3O7), after added a few drops from this reagent appears yellow color precipitate, that’s positive indicated to the presence of alkaloids (Neelima et al., 2011).

3. Fehling's Test

We added 10 ml of copper sulfate and 10 ml of potassium sodium tartrate, then

we weight 5 g of NaOH and add 50 ml of distilled water, then we mix 10 ml of copper sulfate and 10 ml of potassium sodium tartrate with dissolved NaOH. Then we add drpos of NaOH to 10 ml.

Antibacterial activity test

Stock solution (200mg/ml) of aqueous and ethanolic extracts were prepared according to the method in Al Sa'ady (2020). The serially dilutions were prepared from stock solution with sterile distilled water to give three dilutions (200,100, 50) mg/ml. agar-well diffusion method was used to evaluate the antibacterial activity against three species of bacteria included *Salmonella* spp., *Escherichia coli*, *Streptococcus pyogenes*, *Streptococcus pneumonia*, and *Staphylococcus aureus.* A negative control was done by using sterile distilled water. After one-hour pre-diffusion time was allowed at room temperature, plates were incubated at 37°C for 18hr. The inhibition zone was measured in millimeter. It was carried out in duplicates and the mean of the duplicate results were taken.

Microbial test suspensions:

Test organisms used in this study are Gram-positive bacteria (*Staphylococcus aureus*, *Leuconostic mesontroide*), and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*). The microorganisms were maintained on slants of nutrient agar (NA) at 4°C. The inoculums were incubated overnight in nutrient broth at 37°C to produce dense microbial suspension of approximately 106cfu/ml (tube no.6) by diluting fresh cultures and comparing with McFarland density (cells/ml) [18].

Screening for antimicrobial activity:

Hole- plate diffusion method was used for studying the antimicrobial activity and determining the minimum inhibition concentrations (MICs) [19]. Each

inoculum from dense bacterial suspension containing 106 bacterial cells/ml was spread on the surface of Mueller-Hinton Agar. Three holes were made on the media using 6 mm diameter sterile cork-borer. The dried plant alcoholic extract was dissolved in dimethylsulfoxide (DMSO) to provide a stock solution with the final concentration of 4 mg/ml while the plant aqueous extract was dissolved in distilled water to provide a stock solution with the same final concentration of alkoholic extract. A range of serial dilutions were prepared from the stock solution to provide 0.2, 0.4, 0.8, 2, and 4 mg/ml. Each hole diameter (6mm) was filled with (50µl) from diluted of plant extract. The inoculated agar plates were incubated at 37°C for 24 hr. After the incubation period, bioactivity was determined by the measurement of the diameter of inhibition zone around each hole in mm. The inhibition zone was recognized as the area surrounding the hole with no growth of the tested pathogens.

Control plates received only DMSO in Mueller-Hinton Agar without plant ethanolic extract while the control plates in aqueous extract only distilled water in Mueller-Hinton Agar and was run following the same procedure as mentioned earlier. The values reported for diameter of inhibition zone were the average of three replicates

2.2 Result

Table 1 Phytochemical tests of aqueous and alcoholic extracts for phenols reveal the presence of lead acetate, tannin (ferric chloride 1%), while gelatin and saponinm not presence.

Table 1 Phytochemical tests of aqueous and alcoholic extracts for phenols reveal the presence of lead acetate, tannin (ferric chloride 1%), while gelatin and saponinm not presence.

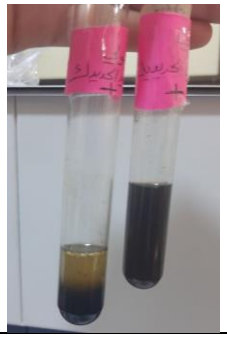
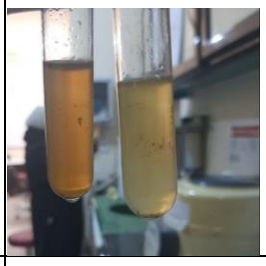
	Test Name	The material to be disclosed	Aqueous Extracts	Alcoholic Extracts	Test Color	Picture
A	**Lead acetate test**	**phenols**	+	+	**Bulky white**	
B	**Tanin test (Frric chloride 1%)**	**Phenols**	+	+	**Black**	
C	**Gelatin test**	**Phenols**	–	–		
D	**Saponin test**	**Phenols**	–	–		

Table 2 phytochemical tests of aqueous and alcoholic extracts for alkaloids reveal the presence of khella, while Mayer's reagents and Hagere's reagents not produce any change.

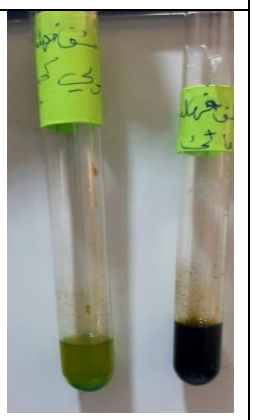
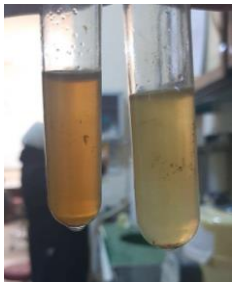
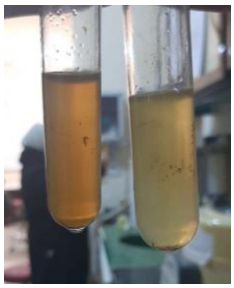
	Test Name	The material to be disclosed	Aqueous Extracts	Alcoholic Extracts	Test Color	Picture
A	**Fehling's Test**	**alkaloids**	**+**	**+**	**Green**	
B	**Mayer's Test**	**alkaloids**	**_**	**_**	**No Change**	
C	**Hagere's Test**	**alkaloids**	**_**	**_**	**No Change**	

Table 3 Antimicrobial activity Ammi visnaga ethanolic extract at various concentrations against pathogenic microbes.

Strain	**DMSO**	**0.2**	**0.4**	**0.8**	**1**	**2**	**4**
Escherichia coli	**0**	**0**	**0**	**6**	**7**	**8**	**12**
Pseudomonas aeruginosa	**0**	**0**	**6**	**8**	**8**	**9**	**9**
Staphylococcus aureus	**0**	**0**	**10**	**12**	**12**	**13**	**15**
Leuconostic mesontroide	**0**	**0**	**0**	**0**	**6**	**8**	**10**

Table 4 Antimicrobial activity Ammi visnaga aqueous extract at various concentrations against pathogenic microbes.

Conc. / strain	**Control (DMSO)**	0.2	0.4	0.8	1	2	4
Escherichia coli	0	0	5	7	8	8	10
Pseudomonas aeruginosa	0	0	0	6	7	8	10
Staphylococcus aureus	0	0	0	0	0	0	0
Leuconostic mesontroide	0	0	0	6	8	9	10

Chapter Three: Discussion

Phytochemical tests of aqueous and alcoholic extracts was used to reveal the presence of phenols and alkaloid by using number of tests, we used lead acetate test that give bulky white precipitate which indicate the presence of phenols, and we used tanin test that give black precipitate which indicate the presence of phenols, in addition we used Gelatin test and Saponin test but does not reveal the presence of phenols. Phytochemical test of aqueous and alcoholic extracts that reveal the presence of alkaloids use another number of tests, the Fehling's test reveal the presence of alkaloids and produce green precipitate while while Mayer's reagents and Hagere's reagents not produce any change.

Stock solution (200mg/ml) of aqueous and ethanolic extracts obtained from Ammi visnaga was tested against (*Staphylococcus aureus*, *Leuconostic mesontroide*), and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*). Hole- plate diffusion method was used for studying the antimicrobial activity and determining the minimum inhibition concentrations (MICs). We made three holes on the media, each hole made with 6 mm diameter and filles with 4 mg/ml of dried plant alcoholic extract that was dissolved in dimethylsulfoxide (DMSO). The bioactivity was determined by the measurement of the diameter of inhibition zone around each hole in mm. A range of serial dilutions were prepared from the stock solution to provide 0.2, 0.4, 0.8, 2, and 4 mg/ml. Each hole diameter (6mm) was filled with (50µl) from diluted of plant extract. Ammi visnaga ethanolic extract showed different antimicrobial activity against the tested pathogens and the diameter of inhibition zone was directly proportional to the increase in plant extract concentration reaching a plateau. As seen in table 3 the inhibited zone increased with increasing the concentration of Ammi visnaga ethanolic extract. *Staphylococcus aureus* and *Leuconostic Mesontroide* require high concentration to

be inhibited. While *Escherichia coli* and *Pseudomonas aeruginosa* require low concentration to be inhibited. The inhibition activity of the plant ethanolic extract on test strains was in decreasing order according to the minimum inhibition concentration as follows: Pseudomonas Aeruginosa < Leuconostic Mesontroide > Escherichia coli > Staphylococcus aureus.

In contrast when we use Ammi visnaga aqueous extract as seen in table 4 the *Staphylococcus aureus* can't be inhibited with any concentration, while *Escherichia coli* require high concentration to be inhibited while the Pseudomonas aeruginosa and the Mesontroide Leuconostic require low concentration of Ammi visnaga aqueous extract to be inhibited. The inhibition activity of the Ammi visnaga aqueous extract on test strains was in equal order with exception of *Staphylococcus aureus* that cannot inhibited.

Chapter Four: Conclusion

Ammi visnaga is an annular plant it is known as Khella, has a slight aromatic odor and a very bitter taste. They have wide medical uses, they have cardiovascular effect, and has vasodilator and bronchodilator activity, and orally, khella is used as antispasmodic for colic and abdominal cramps, kidney stones, menstrual pain, and premenstrual syndrome. In addition, khella topically used for treatment of vetiligo, psoriasis, patchy hair loss (alopecia areata), wound healing, inflammation conditions, and poisonous bites.

The phytochemical tests of aqueous and alcoholic extracts can be used to reveal the presence of alkaloids and phenols, only lead acetate and tanin test (Frric chloride 1%) can be used to reveal the presence of phenols and fehling's test reveal the presence of alkaloids.

The tested ammi visnaga plant extracts exhibit a broad spectrum of activity against various microorganisms. Various concentration of Ammi visnaga ethanolic extract can be used to inhibit various types of pathogens and its inhibition effect was in decreasing order according to the minimum inhibition concentration as follows: Pseudomonas Aeruginosa < Leuconostic Mesontroide > Escherichia coli > Staphylococcus aureus. And also various concentration of Ammi visnaga aqueous extract was used to inhibit various types of pathogenic microbes and its inhibition effect was in equal order with exception of *Staphylococcus aureus* that cannot inhibited.

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