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University of Basra College of Pharmacy

Phytochemical investigation of *Ammi visnaga*

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بسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

(اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ ﴿١﴾ خَلَقَ الْإِنسَانَ مِنْ عَلَقٍ ﴿٢﴾ اقْرَأْ وَرَبُّكَ الْأَكْرَمُ ﴿٣﴾ الَّذِي عَلَّمَ بِالْقَلَمِ ﴿٤﴾ عَلَّمَ الْإِنسَانَ مَا لَمْ يَعْلَمْ)

صدق الله العلي العظيم

Declaration

We hereby declare that this project has been composed by ourselves and has not been submitted in any previous application for a Bachelor's degree. All work presented has been done by us unless otherwise stated. All sources of information have been acknowledged appropriately by means of references.

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Firstly, I would like to express my gratitude and deep appreciation to my supervisors DR. Raafat Mohammed Abdullah and Assistant professor Dr. Ula Almousawi (University of Basra, College of pharmacy) (for their continuous support, encouragement and for evaluating and great advice throughout this work. Also, I would like to thank the deanery of the College of pharmacy represented by the dean Dr. Falah Hussan Shary This work would not be accomplished without support my father and prayer of my mother (may God save them), my lovely sisters, brothers. Finally, my best wishes and thanks are due to our friends and everyone who helped us to complete our study, and not mentioned above.

Abstract :

Medicinal plants are an essential source of human health, so many studies have focused on the use of alternative medicine due to its preventive and therapeutic importance and its benefits in other fields.

Ammi visgana is one of the widely studied It is a treatment for many diseases, including renal colic and coronary artery insufficiency, as well as an antioxidant, antifungal and bacterial agent.

The results of the phytochemical analysis showed the presence of alkaloids, flavonoids, saponins and Carbohydrates.

The ripe fruits and aerial parts of *Ammi visnaga* which is herbaceous plant of Apiaceae family, are collected from northern parts of Iraq in July (Sulaymaniyah), Khellin which is the main constituent of *A.visnaga* belonging to furanochromones groups. Generally had been taken the plant (flower part) to make the study on it after the collection , the drying in the shade and made grinding to it to make the extracted easy .. We had using three solvent for the detection and the extraction depending on solvent. After that the detection for Furanochromone by different chemical method will take it later in the research make sure that the plant is very important for the future.

This study aims to have phytochemical investigation of the main constituents of *A. visnaga* naturally growing in northern part of Iraq harvested during flowering time

Experimental study: The Chemical study was carried out in the Department of Pharmacognosy and Medicinal Plants in College of Pharmacy University of Basra.

1.Introduction :

The use of plants in therapy (phytotherapy) is very old and the public is taking a renewed interest in it. It is possible to use all parts of the plant or the extraction products they provide. The search for new active pharmacological molecules through the screening of natural sources enabled the discovery of a large number of useful drugs that begin to play a major role in the treatment of many human diseases . *Ammi visnaga* it is an annual herbaceous plant with bi-or tripinatisect linear segmented leaves and large compound umbels of white flowers. It grows wild in the Mediterranean region especially in Egypt, Morocco and the Islamic republic of Iran .

It is used locally in traditional medicine. The decoction of the fruits of the plant cures diabetics, treats abscesses, fights diseases that affect the intestines and relieves various pains, including migraine . The khellin , the visnagine and visnadine are the active ingredients of the fruits of *Ammi visnaga* . The chromone Khellin of *A.visnaga* fruit along with visnagin and Khellol glycoside, is a potent coronary vasodilator and bronchodilator and is used for the treatment of coronary insufficiency, angina pectoris and bronchial asthma . The essential oil of khella seems to have some antifungal and antibacterial activity . While its hydro-ethanolic extract is recommended as an effective natural biocide and a potent larvicide against mosquito larvae. This plant constitutes a very important local floristic patrimony.

Figure (1.3) *A.visnaga* field in Sulaymaniyah\ Said sadiq

Photos was taken during the collection of the plant

1.1: Taxonomic hierarchy of *A.visnaga*

Kingdom : Plantae – Plants

Subkingdom: Tracheobionta – Vascular plants

Superdivision : Spermatophyta – Seed plants

Division: Magnoliophyta – Flowering plants

Class : Magnoliopsida – Dicotyledons

Subclass : Rosidae

Order : Apiales

Family : Apiaceae / Umbelliferae – Carrot family

Genus: *Ammi L. – ammi*

Species : *Ammi visnaga* (L.) Lam. – tooth

1.2 Synonym :

1.Selinum visnaga E.H.L. Krause.

2. *Daucus visnaga* L.

3. *Sium visnaga Stockes*.

4.Visnaga daucoides Gaertn.

5.Ammi daucoides Gaertn.

6.Ammi dilatatum St.-Lag.

7.Apium visnaga L

8.Crantz Carum visnaga L.

2.Chemical Constituents :

The chemical constituents of *A. visnaga* are well known and have been reported by many researches in numerous studies throughout the years. Previous studies have reported on various chemical constituents in *A. visnaga*, including γ-pyrones, coumarins flavonoids, and essential oils. The quality and quantity of these secondary metabolites depend on the part of the plant analyzed, as well as the growing conditions and the addition of any bioregulators .

2.1.γ-Pyrones and Coumarins

γ-Pyrones and coumarins are considered to be the major constituents of *A. visnaga*. They include:

γ-Pyrones (Furanochromone Derivatives) Khellin and visnagin are the major ones , in addition to 4-norvisnagin, khellinol, visamminol, ammiol, and khellol . Other important γ-pyrones include 5,7-dihydroxy-2-methyl-γ-pyrone-7-O- glucoside and pimolin (III) , as well as, khellinin, khellinone, and visnaginone .

2.2.Phenolic Compounds

Phenolic compounds are considered an important group of secondary metabolites that has been identified in *A. visnaga*, particularly in its aerial parts, by many researchers.

2.3.**Essential Oil**

Ammi visnaga was found to contain essential oils in different organs, but mainly in the umbels and fruits. Studies used either steam distillation or hydro distillation to

isolate the oil, and then analyses it by means of gas chromatography (GC) and gas chromatography coupled with mass spectrometer (GC-MS) to identify its components. The chemical constituents of the essential oils of *A. visnaga* are distributed mainly among the following groups: no terpenes and monoterpenes , in addition to diterpenes and sesquiterpenes but only in very small amounts in the case of the latter two . The most abundant constituents of steroid and fatty acids**.**

3.Medical uses:

The decoction and/or powdered plant has been traditionally used for the treatment of renal colic, mild anginal symptoms, treatment of abdominal cramps. It is also employed as a supportive treatment for mild obstruction of the respiratory tract in asthma or spastic bronchitis, and postoperative treatment of conditions associated with the presence of urinary calculi. The plant and its extracts are also popular in the treatment of vitiligo and psoriasis, and are used as a lithotripter agent. It is generally used to dilate bronchial, urinary, and blood vessels without affecting blood pressure. It is also internally used as an emmenagogue to regulate menstruation, as a diuretic, and in the treatment of vertigo, diabetes, and kidney stones. An infusion of the aerial parts has also been used to treat headaches .

4. Material and Methods:

4.1 Instruments:

Calibration of instrument and lab tools are done according to supplier instructions. The glassware's are washed by distilled water and rinsed by acetone then dried before every use. The instruments used are shown below:

1-Electrical sensitive balance

2-Hot plate

3-Micropipette

4-Cylinder

5-Funnel

6-Beaker(250ml)

7-Distillation flask

8- Soxhlet device

4.2Chemical and material:

Chemicals where in analytical grade, unless otherwise specified. Chemicals and material used are listed below :

1-The major solvent (Chloroform , Acetone and Ethanol)

2-potassium bismuth iodide

3-potassium mercuric iodine solution

4- -magnesium ribbon or magnesium metal

5-concentrated hydrochloric acid (HCL)

6-neutral ferric chloride solution (ferric chloride test)

7-distilled water

8-sulphuric acid

9-α-naphthol

10-Ethanolic m-dinitrobenzene

4.3 Plant collection :

Samples collection: An intensive survey was carried out during the summer OF July for the collection and identification of plants. It was carried out in Sulaymaniyah .Collection of *A.visnaga* was done in the north of Iraq since its presence common in this part of the country, to obtain the ripe fruits of *A.visnaga* for collection was done during July.

4.3.1 drying :

Arial Plant parts were washed carefully to avoid losing materials then undergo air-drying in the shade for several days at room temperature . Drying was done in the shade to avoid losing the volatile oil and to remove the water so decrease the chance of fermentation of the plant. The pigmentation effect of the plant must be taken in mind and the plant must be handled carefully by using gloves and washing the hands after that.

4.3.2 Storage:

Arial parts of *A. visnaga* were divided into parts and stored in a plastic labeled container with descants of silica gel bags to remove humidity that could accelerate enzymatic degradation of the active constituent.

5. Extraction of *AMMI. VISNAGA:*

Dried aerial parts of *A.visnaga* were grinded by mechanical grinder to obtain fine powder in order to increase the surface area and speed of extraction. The plants were then dried undergo air-drying in the shade and had been using three solvent then for the extraction below:

1. 20 gram of powdered *A. visnaga* were transferred into 33*100mm extraction thimbles. Using Soxhlet device using 100 ml to 200 ml of chloroform as a solvent till the color in the draining arm became clear that lasted for us 9 hours In complete to be clear with temp. of device (75 °C). Chloroform extract was stored in dark container for further investigation for detection.

2. 20 gm dry plant material was dried under the hood then extracted with 100 ml Acetone by Soxhlet till the color in the siphon arm became Clear so that take us 9 hours in complete to be clear with temp. of the device from (66 °C) and stored in dark container in refrigerator for further investigation for detection .

3. 20 gm dry powder plant material was dried under the hood then extracted with 200 ml Ethanol by Soxhlet device till the color in the siphon arm became clear that been for 9 hours extract with temp. (88 °C) The final volume was reduced to 150 ml by evaporation and stored in dark container in refrigerator for further investigation for detection.

5.1 Phytochemical screening by chemical tests

We identified the active constituents using standard procedures. By performing chemical tests on the extracted plant material as follows:-

1-Test for Alkaloids

The various extracts were basified with ammonia and extracted with chloroform. The chloroform solution was acidified with dilute hydrochloric acid. The acid layer was used for testing the alkaloids. The following two tests were used for detection of alkaloids…

A.Dragendroffs test: (potassium bismuth iodide) Had been used for the three extract with the same procedure that we take 1 ml after diluted it with ethanol to decrease its conctreated from each extract in pipette and put it in the tube The acid layer was treated with few drops Dragendroffs reagent(about 2 to 3 drops). Formation of reddish brown precipitate indicated the presence of alkaloid…

B-Mayer's test: (potassium mercuric iodine solution): The second test for alkaloid detection also take 1 to 2 ml from each diluted extract and put it in the tube The acid layer was treated with few drops about 3 to 5 drops of Mayer's reagent. Formation of creamy white precipitate indicated the presence of alkaloids.

2. Test for flavonoid test : we had done two test

A-Shinoda test: take 2 ml from each diluted extract with alcoholic solution and put in tube with a few fragments of magnesium ribbon (1 to 2 ribbon)and a few drops of concentrated hydrochloric acid were added…... Appearances of red to pink color after few minutes indicated the presence of flavonoid.

B- Ferric chloride test: few drops of neutral ferric chloride solution were added to little quantity of alcoholic extract for each one taken 1 ml except acetone taken 2ml. Formation of blackish green color indicated the presence of flavonoids.

3- Test for tannins The following test was used for detection of tannins

A-Ferric chloride test: 1ml of diluted extracts with few drops of 1% neutral ferric chloride solution was added. Formation of blackish blue color indicated the presence of tannins.

4-Test for saponins

A- Foam test: small amount of each diluted extract(1ml) was shaken with little quantity of distilled water, if foam produce persist for 10 minutes, it indicated presence of saponin.

5-Test for Carbohydrates

Small amount of extracts/ fractions were dissolves in little quantity of distilled water and filtered separately. The filtrates were used to test presence of carbohydrates.

A- Molisch's test: (general test for the presence of carbohydrate): To 1 ml of diluted extracts 2 drops of α-naphthol was added and 2ml concentrated sulphuric acid was poured carefully down the side of the test tube. Formation of purple ring at interface of two layers indicated presence of carbohydrates.

6-Horstmann's m-dinitrobenzene detection:

The Horstman’s reagent consist of :

(A)2 gram of Meta dinitrobenzene dissolved in 100ml 95% ethanol

(B) 0.5 ml of 50% potassium hydroxide Samples were transferred in test tube with the addition of solution (A) followed by solution (B). We using 2 ml for each extract Appearance of violate color indicates the presence of furanochromone.

5.Results and Discussion:

5.1 plant collection of *A.visnaga*

was done in the north of Iraq since its presence common in this part of the country, to obtain the ripe fruits of *A.visnaga* collection was done during July. Drying was done in the shade to avoid losing the volatile oil and to remove the water so decrease the chance of fermentation of the plant. The pigmentation effect of the plant must be taken in mind and the plant must be handled carefully by using gloves and washing the hands after that.

5.2 Extraction of *AMMI. VISNAGA*

Extraction of active constituents are the most important step in the analysis of medicinal plants, since it allows us to obtain the desired substance for further evaluation. Basically extraction starts with steps like pre-washing, drying, and grinding of plant material to obtain fine homogenous powder to increase the speed of extraction by increasing the surface area that face the extracting solvent. The choice of the extraction method depends on the nature of the desired substance to be isolated and analyzed like its solubility, polarity and thermal stability. Different methods in past researches are used for the extraction of *A. visnaga* active constituents using different solvents like chloroform, acetone

The weight of the yield for each solvent we using in the project

1.first solvent is chloroform how had nonpolar property with B.P about (61.2 °C)(142.2°F

For nonpolar chloroform the yield weight for the pure extract by chloroform is 3.2021 gm.

2.The second solvent is Acetone as medium solvent has both polar and nonpolar property

(semipolar) The boiling point of acetone is 56.05°C (132.8°F) at standard atmospheric pressure.

the yield weight for the pure extract by Acetone is 2.5117 gm.

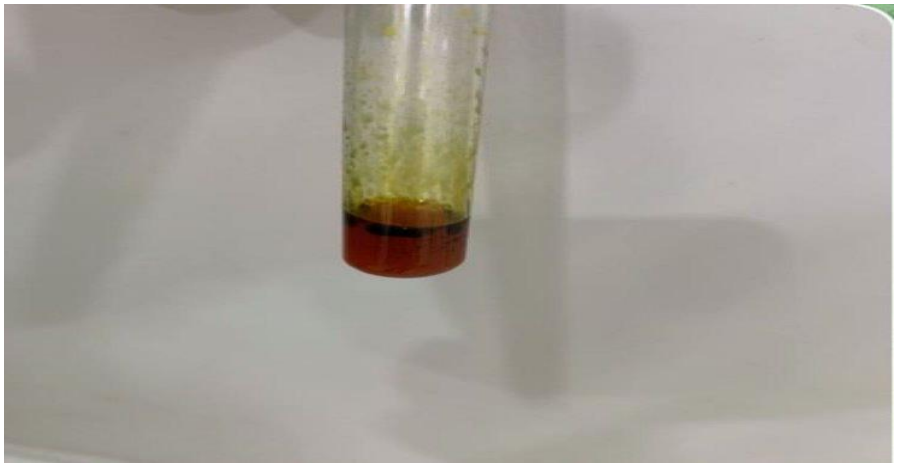
3. the final solvent extract is Ethanol a polar solvent with boiling point of ethanol is 78.37°C

(173.1°F) at standard atmospheric pressure. the yield weight for the pure extract by Ethanol is 4.1105gm. (it has higher weight than another extract because of it's ability to dissolve large amount of compound from the crude plant because of the it high polarity).

5.3 Phytochemical screening by chemical test

5.3.1 1-Test for Alkaloids

A. Dragendroffs test: (potassium bismuth iodide) : Formation of reddish brown precipitate indicated the presence of alkaloid….. All chloroform .Acetone and ethanol has +ve result of presence of alkaloid.



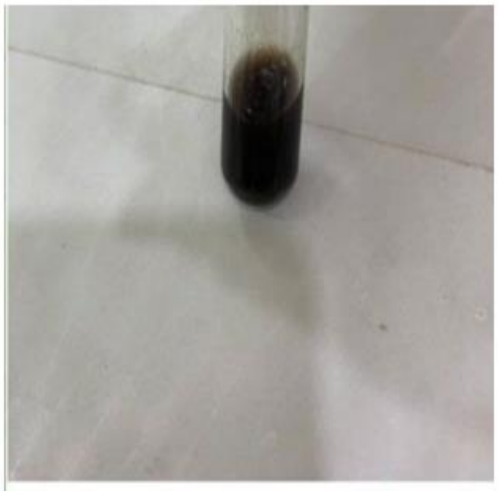
B-Mayer's test: (potassium mercuric iodine solution):

Formation of creamy white precipitate indicated the presence of alkaloids. All the solvent extract +ve result detection especially for acetone and ethanol . a weak result positive for chloroform meaby because of little turbid it it's diluted.

2. Test for flavonoid test:

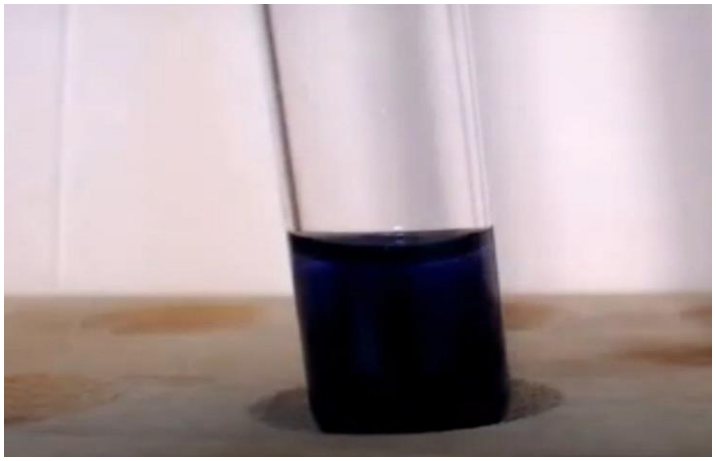
A-Shinoda test: All the tests Appearances of red to pink color after few minutes indicated the presence of flavonoids …

B- Ferric chloride test: all extract Formation of blackish green color indicated the presence of flavonoids. Chloroform is weak +ve



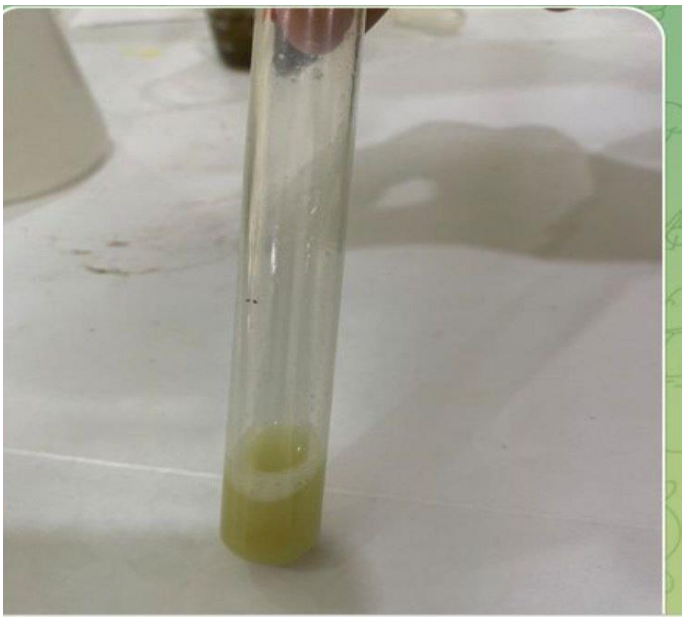
3- Test for tannins:

A-Ferric chloride test: Formation of blackish blue color indicated the presence of tannins. All of the extract +ve result detection of tannin in this test.



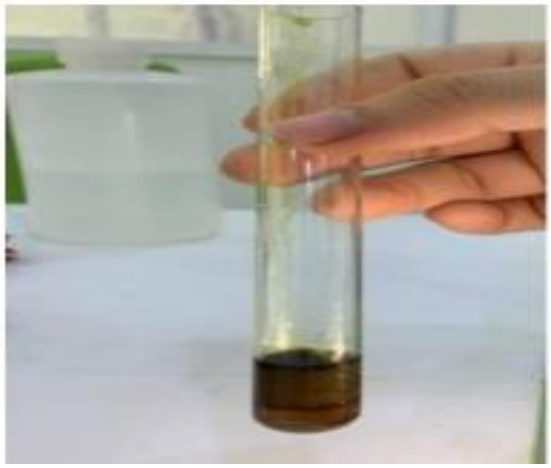
4- -Test for saponins :

A- Foam test: if foam produce persist for 10 minutes, it indicated presence of saponin. Both Ethanol an chloroform was +ve test but acetone was weak positive.

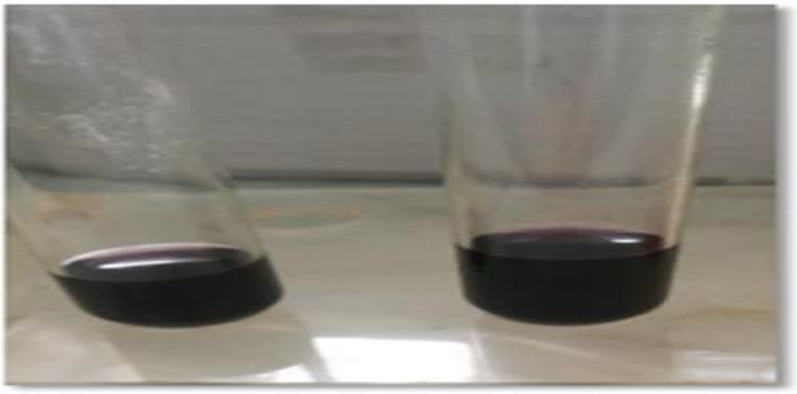


5-Test for Carbohydrates:

A- **Molisch's test**: Formation of purple ring at interface of two layers indicated presence of carbohydrates… All the extracted test +ve test .



6-Horstmann's m-dinitrobenzene detection: Appearance of violate color indicates the presence of furanochromone . All the test extract +ve result of furanochromone .



Active constituent	Type of test	Chloroform extract	Acetone extract	Ethanol extract
1.Alkaloids	Dragendroff's test	+	+	+
Alkaloids	Mayer's test	Weak +	+	+
2.flavonoids	1.Shinoda test	+	+	+
	2.Ferric chloride test	Weak +	+	+
3.Saponin	Foam test	+	Weak nearly to _	+
4.Carbohydrates	Molisch's test	+	+	+
5.furanocoumarins, such as khellin and visnagin	Horstmann's m-dinitrobenzene detection	+	+	+
6.Tannin test	Ferric chloride test	+	+	+

Conclusion :

1.Khellin is the main furanochromone of *A.visnaga* grown in the northern parts of Iraq extracted and isolated from the fruit and Aerial parts by simple and reproducible methods.

2. Phytochemical analysis of *Ammi visnaga* extracts has also revealed the presence of other compounds, including flavonoids, volatile oils, and phenolic acids, which may contribute to the plant's medicinal properties. However, it is important to note that the use of *Ammi visnaga* should be guided by a healthcare professional, as the plant may cause adverse effects in some individuals.

3. In conclusion, the extraction and detection of phytochemicals in *Ammi visnaga* have been studied extensively due to the plant's medicinal properties. Several methods have been employed to extract the active constituents from the plant, including maceration, Soxhlet extraction, and ultrasound-assisted extraction. These methods have been shown to yield extracts with high levels of furanocoumarins, flavonoids, volatile oils, and phenolic acids. IN our project we using the chemical test method for work.

Recommendations:

1.continued research on Ammi visnaga is necessary to further explore its potential as a therapeutic agent and to ensure its safe and effective use in medicine.

2.research on the cultivation, extraction, and processing of Ammi visnaga may also be valuable to ensure the consistency and quality of the plant extracts used in research and medical applications.

3.further research is needed to fully understand the mechanism of action and safety of Ammi visnaga and its active constituents. This includes studies on the pharmacokinetics and pharmacodynamics of the plant, as well as its potential interactions with other medications.

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