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Phytochemical Screening Of An Umbelliferae: *Ammi visnaga* L. (Lam.) In The Region Of Sidi Slimane- North-West Of Morocco

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Abstract

Ammi visnaga L. (Lam.) is an herbaceous, Mediterranean, annual plant in the Apiaceae family, present in a spontaneous state in Morocco on a clayey soil. In order to enhance the aromatic and medicinal plants of Morocco, we focused on the characterization as well as the phytochemical identification of the Ammi visnaga, which is widespread in the region of Sidi Slimane (North-West). In this context, qualitative analysis of *Ammi visnaga* seeds and dry umbels was performed by phytochemical screening which is based on staining and precipitation reactions by specific reagents, as well as confirmatory tests by TLC. Therefore, several extraction solvents of different polarity were used. The phytochemical screening of *Ammi Visnaga L. (Lam.)* revealed the presence of several classes of secondary metabolites namely Polyphenols, Tannins, Flavonoids, Alkaloids, Reducing compounds, Cardiac glycosides, Catechols, Sterols and Terpenes, Coumarins, Quinones , Mucilage, Essential Oil, C-Heterosides and O-Heterosides.

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 [1]. 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 [2]. This study focuses on one of the species of the Apiaceae/Umbelliferae family: *Ammi visnaga* (Khella) with synonyms Ammi daucoides and Daucus 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 [3].

In Morocco, *Ammi visnaga* is a very abundant spontaneous plant, particularly in the northern region. 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 [4]. The khelline, the visnagine and visnadine are the active ingredients of the fruits of *Ammi visnaga* [5]. The chromone Khellin of 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 [6]. The essential oil of khella seems to have some antifungal and antibacterial activity [4-7]. While its hydro-ethanolic extract is recommended as an effective natural biocide and a potent larvicide against mosquito larvae [8]. This plant constitutes a very important local floristic patrimony, but few studies have been done on this species which has never been studied before in the region of Sidi Slimane located at 34 ° 16' North, 5 ° 55' West of Morocco.

The present study aims at demonstrating the richness of the seeds and dry umbels of *Ammi visnaga L. Lam.* Its containing, particularly phenolic compounds, nitrogen compounds, terpene compounds, and many other compounds which may be the origin of important therapeutic properties.

In this respect, our study encompasses two aspects:

The first aspect is phytochemical that is based mainly on a qualitative study, comprising a phytochemical screening, which is, in turn, founded on coloration and precipitation reactions.

The second aspect is devoted to confirm these results through the chromatographic identification by TLC of the main secondary metabolites which may exist in this plant.

2. Material and Methods

2.1. Plant material

The umbels of *Ammi visnaga (L.) Lamarck* (Apiaceae) were collected in the region of Sidi Slimane (northwestern Morocco) in August (2015). The various organs (seeds and umbels without seeds) immediately cut and separated from each other. After fragmentation in a mortar, they were powdered and stored in a dry shelf protected from light.

2.*2. Sample Preparation*

The following extracts were prepared according to each selected organ:

Aqueous extract: 1 g of vegetable powder was introduced into 20 ml of boiling distilled water and infused for 15 minutes, then filtered and rinsed with a little hot water so as to obtain 20 ml of filtrate.

Ethyl acetate extract: 1 g of plant powder is introduced into 20 ml of ethyl acetate and macerated for 24 hours. The extracts obtained are stored at 4 °C and protected from light until they are used.

2.3. Phytochemical Screening

Secondary metabolite characterization tests involve qualitative analysis either on the formation of insoluble complexes using precipitation reactions or in the formation of colored complexes using staining reactions.

2.3.1. Characterization of phenolic compounds

a. Polyphenols

The reaction with ferric chloride ($FeCl_3$) made it possible to characterize the polyphenols. 2 ml of each extract was added a drop of 2% solution of methanolic ferric chloride. The appearance of either darker blue-black or green color was a sign of the presence of polyphenols [9].

b. Flavonoids

Flavonoids, which are virtually universal pigments of plants, constitute a large family of compounds abundantly present in plants [10].

- *Reaction to Cyanidin*

5 ml of hydrochloric ethanol (95% ethanol, distilled water and hydrochloric acid R in equal volumes of 5 ml) was added to 5 ml of each extract (aqueous, ethyl acetate) with a piece of magnesium ribbon as well as 1 ml of isoamyl alcohol.

- A pink-purple color indicates the presence of flavanones.
- A red color indicates the presence of flavonols and flavanonols.

The reaction with cyanidine is carried out without the addition of metallic magnesium and heated for 10 minutes in a water bath. In the presence of leucoanthocyanins, a red cherry or red color is developed; the catechols give a red-brown tint [10].

- *Anthocyanins*

5 ml of each extract (aqueous, ethyl acetate) was added to 5 ml of sulfuric acid (H_2SO_4) and 5 ml of ammonium hydroxide (NH_4OH). A red coloration in acidic medium and blue-purple in a basic medium testifies to the presence of anthocyanins [10].

c. *Tannins*

1 ml of each extract was added in 1 ml of water in a test tube. 2 to 3 drops of 1% diluted ferric chloride solution was added. A dark green or blue green coloration indicates the presence of tannins.

- The appearance of a dark green color indicates the presence of catechic tannins.
- The appearance of a green blue color indicates the presence of gallic tannins [10].

d. Coumarins

5 ml of the ether extract was evaporated to dryness. The residues are taken up in 2 ml of hot water and 1 ml of 25% NH_4OH. The observation under UV at 366nm of an intense blue fluorescence indicates the qualitative presence of coumarins [10].

2.3.2. Characterization of nitrogen compounds- Alkaloids

The characterization of the alkaloids was carried out by precipitation reactions with the reagents of Dragendorff and Mayer.

- Dragendorff test: 1 ml of each extract was placed in test tubes with 5 drops of Dragendorff reagent, the appearance of an orange precipitate, reveals the presence of alkaloids [11].
- Mayer test: 1 ml of Mayer's reagent was added drop by drop to each extract. The formation of a greenish-colored precipitate or cream indicates the presence of alkaloids [12].

2.3.3. Characterization of Terpenic Compounds

a. Carotenoids

2 to 3 drops of a solution of saturated antimony trichloride in chloroform were added to the 5 ml residue of the dry evaporated macerate. The development of a red color turning red indicates the presence of carotenoids [13].

b. Sterols and Triterpenes

The ether extract is prepared from 1 g of vegetable powder in 20 ml of ether for 24 hours. The sterols and triterpenes are identified by adding 1 ml of $CHCl_3$ to the residue of 10 ml of the evaporated macerate. The solution obtained is divided into two test tubes and then 1 to 2 ml of concentrated H_2SO_4 is added to the bottom of one of the tubes and the other will serve as a control. The formation of a red-brown or purple precipitate reveals their presence [13].

c. *Essential oils*

1 g of plant material is introduced into 10 ml of dichloromethane and the extract is evaporated to dryness. The residue is dissolved in 3 ml of ethanol. The solution was evaporated again to dryness. The sensation of a scented odor indicates the presence of essential oils [14].

2.3.4. Charcterization of Heterosides

a. Saponins

1 g of the powdered plant material placed in 100 ml of boiling distilled water makes it possible to obtain 1% of a decoctate. The latter is then placed in the water bath for 15 minutes at a boiling point. The decoctate is then filtered and distributed in 10 test tubes of 1 to 10 ml in a sequential manner. The volume of each tube is supplemented to 10 ml with distilled water. Each tube is stirred vertically for 15 sec. The height of the foam is then measured after 15 minutes of rest. The foam index is calculated at the level of the tubes where the height of the foam is greater than or equal to 1 cm according to the following relation:

Im = 1000 / N (N: tube number in which the foam is 1 cm) [15].

b. Cardiac glycosides

2 ml of chloroform was added to 1 ml of each extract, the appearance of reddish brown coloration after addition of H_2SO_4 indicates the presence of cardiac glycosides [10].

c. Cyanogenetics

3 g of plant material was wetted with a few drops of chloroform ($CHCl_3$) in a test tube, into which a strip of filter paper impregnated with sodium picrate was inserted. The tube is then placed in a water bath at 35 °C for 3 hours. A red band turn indicates the presence of cyanogenic compounds (by production of HCN) [16].

d. Anthracene derivatives

The anthracene derivatives are found in plants, in the form of free or combined genes of quinine (or aglycones) of anthracene heterosides.

The presence of the anthracene derivatives is demonstrated by means of the 50% diluted NH_4OH solution present in the reaction mixture.

A chloroform extract is prepared from 1 g of sample powder in 10 ml of $CHCl_3$ and then heated for 3 minutes in a water bath. The solution is filtered hot. The residue of the powder exhausted by $CHCl_3$ will serve to demonstrate the heteroside forms (O-heterosides and C-heterosides).

-Free anthracene derivatives

The free anthracene derivatives are demonstrated by adding 1 ml of dilute NH_4OH solution to 1 ml of the chloroform extract, followed by stirring. The appearance of a more or less red color indicates their presence [17].

-Combined anthracene derivatives

O-heterosides: Characterized by adding to the residue the previously depleted $CHCl_3$ powder, 10 ml of distilled water and 1 ml of concentrated HCl. The whole is then heated for 15 minutes in a water bath, then cooled under a stream of cold water and filtered and made up to 10 ml with distilled water. 5 ml of the filtrate are stirred with 5 ml of chloroform.

The organic phase is then withdrawn and introduced into a test tube in which is added 1 ml of dilute NH_4OH. The presence of anthraquinones is revealed by a more or less intense red color. If the reaction is negative or weakly positive, the characterization of the O-heterosides at reduced genes is demonstrated.
C-heterosides: characterized by adding 10 ml of water and 1 ml of 10% solution of $FeCl_3$ to the aqueous phase obtained previously with O-heterosides, the mixture is heated for 30 minutes in a water bath and Cooled under a stream of water. The organic phase is separated after stirring with 5 ml of $CHCl_3$ and 1 ml of dilute NH_4OH. The appearance of the red coloration more or less intense indicates the presence of C-heterosides [18].

2.3.4. Characterization of Reducing Compounds
Their detection consists of treating 1 ml of each extract with 2 ml of distilled water and 2 ml of Fehling solution and then the tubes are incubated in a Marie bath at 40°C. A positive test is revealed by the formation of a brick Red precipitate [19].

2.3.5. Characterization of Free Quinones
1 g of plant powder is placed in a test tube to which 15 to 30 ml of petroleum ether are added. After stirring, the mixture is left to stand for 24 h. The extracts are filtered and evaporated to dryness. The presence of free quinones is confirmed by a yellowing of the aqueous phases following the addition of a few drops of 1/10 NaOH [19].

2.3.6. Characterization of Proteins
Proteins are identified in aqueous and acetate residues by the biuret reaction. To an aliquot of the residues, 2 ml of 20% aqueous NaOH was dissolved in a test tube and then 2 to 3 drops of a 2% aqueous $CuSO_4$ solution were added. The appearance of a purple coloration, sometimes with a reddish hue, indicates a positive reaction [20].

2.3.7. Characterization of Mucilages
1 ml of 10% decocted extracts is added to 5 ml of absolute ethanol. The formation of a foamy precipitate indicates the presence of mucilage [15].

2.3.8. Characterization of narcotic drugs -Tetrahydrocannabinol (THC): Reaction of Bean
0.5 g of the powder was introduced into a test tube with 5 ml of petroleum ether and then stirred for 15 minutes. The ethero-petroleum phase is then decanted in a capsule and evaporated to dryness in a water bath. 3 to 4 drops of 5% KOH was added to the alcohol. A purple color indicates a positive reaction [17].

2.4. *Thin Layer Chromatography TLC*
For a partial characterization of the various extracts of umbels and seeds of *Ammi visnaga*, thin layer chromatography (TLC) is carried out. The support, used in this study, is a silica gel plate (20 × 20 cm, 60 F254), developed by different migration solvents. After drying, the chromatograms were visualized under UV / 366 nm. The solvent systems used are summarized in Table 1 below:

Table 1: Thin layer chromatography and extraction solvents

Secondary metabolite	Extraction solvents	Migration solvents
Coumarins [21]	Chloroform	Ethyl acetate / Toluene (7 : 93) ml
Flavonoids [22]	Methanol	Ethyl acetate / Methanol/ H_2O (100: 13,5: 10) ml
Tannins [23]	Acetone	Ethyl acetate / Methanol/ H_2O (40 : 8 : 5) ml
Anthraquinone [24]	Methanol	Ethyl acetate / Methanol / H_2O (81 : 11 : 8) ml
Saponins [25]	Methanol	Chlorofome / Methanol /H_2O (81 :11 : 8) ml
Terpenoids [21]	Hexane	Benzene

3. Results and discussion

3.1. Characterization of secondary metabolites by staining and precipitation reactions
The results of the phytochemical screening carried out are reported in Table 2 according to the degree of reactivity.

Table 2: Combination of chemical characterization results

Chemical groups				Extraction Solvent	Umbels	Seeds
Phenolic compounds	Polyphenols			Aqueous	+	+++
				Ethyl acetate	+	+++
	Flavonoids	Anthocyanins		Aqueous	-	-
				Ethyl acetate	-	-
		Cyanidine	Flavonols	Aqueous	++	+++
				Ethyl acetate	+	+++
			Leucoanthocyanins	Aqueous	-	-
				Ethyl acetate	-	-
			Catechols	Aqueous	++	+++
				Ethyl acetate	+	-
	Tannins	Simple tannins ($FeCl_3$)		Aqueous	+++	+++
				Ethyl acetate	+	+++
		Gallic. T		Aqueous	-	-
				Ethyl acetate	-	-
		Catechic. T		Aqueous	-	-
				Ethyl acetate	-	-
	Coumarins			Ether	+++	++
Nitrogen compounds	Alkaloids	Dragendorff		Aqueous	-	+++
				Ethyl acetate	-	+++
		Mayer		Aqueous	-	+++
				Ethyl acetate	-	+++
Terpenic compounds	Carotenoids			Aqueous	-	-
				Ethyl acetate	-	-
	Sterols and Triterpenes			Ether	+++	+
	Essential oils			Dichloromethane	+++	+
Heterosides	Saponins			Aqueous	-	-
				Ethyl acetate	-	-
	Cardiac glycosides			Aqueous	++	+++
				Ethyl acetate	+++	+++
	Cyanogenetics			Chloroform	-	-
	Anthracene derivatives	Free Anthracenics		Chloroform	-	-
		Combined Anthracenes	O-heterosides	Chloroform	-	+
			O-heterosides at reduced Genes	Chloroform	+	+++
			C-heterosides	Chloroform	+++	+++
Reducing compounds				Aqueous	+	+++
				Ethyl acetate	-	-
Quinons				Petroleum ether	++	+
Proteins				Aqueous	-	-
				Ethyl acetate	-	-
Mucilages				Aqueous	+	+
Narcotics				Aqueous	-	-

+++: Strongly positive; ++: rather positive; +: Slightly positive; -: Negative

The polyphenols were detected in the umbels, in the form of traces opposite to the seeds which show a strongly positive reaction in two extracts. According to the obtained staining with $FeCl_3$, the simple tannins are widely present in the aqueous extract of the umbels and seeds. Also in the acetate extract of the seeds while they are in the trace state in the acetate extract of the umbels. Gallic and catechic tannins are nonexistent in all the extracts. The reaction with cyanidin confirms the presence of flavones at the level of the seeds in the various extracts of large quantity, unlike the umbels. Yet, it contains no anthocyanins and leucoanthocyanes.

The Dragendorff reagent indicated the presence of alkaloids at the seeds level by the appearance of precipitate orange and a white precipitate by the Mayer reagent in the aqueous acetate extract, in contrast to the extracts of the umbels which, do not contain. The reducing compounds are generously present in the aqueous extract of the seeds opposite the umbels which exhibits a slightly positive reaction. A complete absence of these compounds was found in the acetate extract of the two parts of the plant.

The aqueous and acetate extracts of seeds and umbels showed richness in cardiac glycosides. Sterols and terpenes, coumarins and quinones are present predominantly in umbels rather than in seeds. As for Mucilages, essential oils, C-heterosides and O-heterosides with reduced genes; they are present approximately in both parts. Finally, the absence of Saponins, narcotics, carotenoids, cyanogens, free anthracene derivatives and proteins in the two parts studied is noteworthy.

The results of the characterization of substances extracted by water are grouped in the following table:

Table 3: Substances extractable by water

	Weight of empty beaker P1 (g)	**Beaker weight after evaporation P2 (g)**	**P2-P1(g)**	**Percentage of extractable substances per water (%)**
Umbels	47.853	47.916	0.063	6.3%
Seeds	51.526	51.609	0.083	8.3%

According to the table above, the percentage of substances extracted by water in seeds is higher than umbels.

3.2. Results of chromatographic tests

Phytochemical screening by staining and/or precipitation reactions does not provide information on the nature of the chemical molecules, so that further confirmatory chromatography experiments are used (see Table 4).

Table 4: TLC Results

Phytochemical compounds	**Umbels**		**Seeds**	
Coumarins	Rf	Color	Rf	Color
	0.184	Purple	0.142	Purple
Flavonoïds	-	-	0.833	Purple
Tannins	0.918	Purple	0.917 0.751 0.639 0.488 0.263	Purple Purple Purple Purple Purple
Anthraquinone	0.831	Purple	0.838 0.538 0.384	Purple
Saponins	0.977	Purple	0.955	Purple
Terpenoids	0.932	Purple	0.936	Purple

The table 4 reports the characterization of the different groups of secondary metabolites as a function of the distinct UV / 366 nm colorations. The various chromatographic profiles show that the methanolic extracts are rich in flavonoids, anthraquinones and saponins; chloroformic acid in coumarins; acetone to tannins and hexanic acid to terpenoids. The spots observed on the chromatograms of characterization of tannins and anthraquinones at the level of the seeds may correspond to several classes of secondary metabolites. Thus, we could identify in the extracts studied, all the chemical compounds sought (coumarins, flavonoids, tannins, anthraquinones, saponins and terpenoids).

The phytochemical tests carried out on the various extracts studied of the seeds and umbels of *Ammi visnaga L. Lam* were positive with a multitude of the desired compounds.
The results of our study are confirmed by the work of **Soro K et al., 2015** [26], concerning Alkaloids, Flavonoids, Mucilages, Sterols and Terpenes, **Amin et al., 2015** [27] for polyphenols, tannins, cardiac glycosides and absence of saponins, **Zoubi Y et al, 2016** [8] for flavonoids, tannins, sterols and terpenes, coumarins, mucilages and glycosides. However, according to **Soro, KN et al., 2015**[26] and **EZ Zoubi Y et al, 2016** [8], catechic tannins gave positive reactions in the powder of fresh umbels and leaves, stems and roots of *Ammi visnaga*, these were absent in the analyzed samples.

This Mediterranean plant is rich in furanchoromones such as khelline and visnagine, coumarins and flavonoids [28]. Two flavonols (quercetin and kaempferol) were identified in *A. visnaga* growing in Iraq [29]. Although we note the richness of our plant in catechols, coumarins, quinones, C-heterosides and O-heterosides, we also note the complete absence of proteins, free anthracene derivatives, cyanogenetics, carotenoids, reducing compounds, gallic and catechic tannins, Anthocyanins and Leucoanthocyans. **Martelli et al., 1984** [30] and **Eldomiaty, 1992** [31] revealed the presence of various groups of chemical constituents such as pyrones, saponins, flavonoids and essential oils. An extract of khella appears to have antimicrobial activity. This may be due to both khellin and visnagine components, both of which appear to have antifungal, antibacterial and antiviral activity [32].

This abundance of active ingredients with various pharmacological properties gives the plant remarkable properties which could justify its multiple therapeutic indications and its use in traditional medicine. An exploitation of its pharmacological properties implies a further investigation of its active ingredients, currently under way.

Conclusion

The discovery by qualitative tests of the main secondary metabolites synthesized by *Ammi visnaga* L. (Lam.) from the Apiaceae family collected in the region of Sidi Slimane in the month of August 2015 revealed the presence of polyphenols, tannins, Flavonoids, alkaloids, reducing compounds, cardiac glycosides, catechols, sterols and terpenes, coumarins, quinones, Mucilage, essential oil, C-heterosides and O-heterosides. The results of this study reveal that this gasoline possesses a range of potentially bioactive substances able to be exploited on several scales (pharmaceutical, food, cosmetics ...).

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