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Phytochemical and Antioxidant Activity of the Essential Oil of *Ammi visnaga* L. *from* Morocco

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ABSTRACT

The objective of this work is the valorization of Ammi visnaga L. by chemical characterization, as well as the study of the antioxidant activity of its essential oil. The extraction of essential oil from seeds and umbels of this plant is carried out by hydrodistillation, and yields are 0.26% for dry umbels, 0.14% for fresh umbels, 0.02% for dry seed, while the dried umbel in an oven had a yield of 0%. The analysis of the essential oil of A. visnaga by Gas chromatography-Mass spectrometry (GC-MS) identified 29 principle compounds (Campholenal (13.6%)), longipinene (5.53%), longifolene (5.48%), estragole (6.53%) and β-Mentha 1-b-8-ol (25.57%). Furthermore, the antioxidant activity of this essential oil was evaluated by the method of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and showed a significant efficiency in radical DPPH reducing with an IC_{50} value of essential oil of 56.053 ± 0.856 µg/ml.

Keywords: *Ammi visnaga*, Essential oil, GC-MS, Hydrodistillation, Chemical composition, Antioxidant activity

INTRODUCTION

Medicinal and Aromatic Plants (MAP) represent a considerable economic interest in perfume, cosmetics and food industries for their antioxidant activities and flavoring and in pharmacy through their antiseptic, analgesic, anti-inflammatory and antispasmodic properties [1]. Indeed, they constitute an inexhaustible reservoir of folk remedies and a natural source of most currently used drugs [2]. Essential oils (EO) from plants have a plurality of properties mainly due to their complex chemical composition [3]. These oils have a very broad spectrum of action since they inhibit both the proliferation and synthesis of bacterial toxins and yeast, they act on the biomass and production of pseudo mycelium and mold, they inhibit the spore germination, the mycelium elongation and toxin production. EOs also has antiviral, immunostimulant, anti-inflammatory, analgesic actions and stimulating gastric motility [4]. Currently they are studied to better determine their effectiveness as natural preservatives. Through its geographical position, Morocco is characterized by an ecological diversity, which results in a great diversity of flora and a high rate of endemism [5]. Morocco is a traditional producer of MAP; it is one of the leading worldwide suppliers of rosemary, verbena, coriander, pennyroyal, thyme and lavande etc., and an exclusive supplier of several EOs as wormwood, wild chamomile and annual Tansy. Morocco also has an ancestral knowledge of medication by plants, their use for flavoring and preserving food [6].

Ammi visnaga L is a medicinal plant of Mediterranean origin. It's an herbaceous annual plant, with small white flowers, spontaneous, thermophilic, belonging to the family Apiaceae [7]. Its use in traditional medicine dates back to Pharaonic times, this plant, apart from its antispasmodic properties, is used either by fumigation or decoction of its umbel and its fruits or by distillation of its seeds [7]. A decoction of the fruit of the plant treats oral abscesses and gum disease, and fight against diseases affecting the intestines [8]. This decoction is taken orally, against the aortic palpitations, kidney pain and bladder, and also relieves diabetics. In fumigation, fruits and umbels serve to dispel dizziness and headache. The rays of umbels that converge after flowering take shelter in time and are used as toothpicks. Extracts from seeds of this plant are used in medicine for the treatment of coronary diseases and bronchial asthma. Khellin the visnagin and visnadin are the active ingredients of the fruits of *A. visnaga*. They are well used in pharmaceutical industry [9-11].

The essential oil of *A. visnaga*, has tremendous significance *in vitro* inhibitory activity against bacteria and fungi [7]. In this context, the objective of this study is to demonstrate the antioxidant activity of essential oil of *A. visnaga* collected from Taounate region of Morocco, and to study its chemical composition by chromatographic and spectroscopic analysis.

MATERIALS AND METHODS

Plant material

Samples of *Ammi visnaga*, were collected from Taounate region in Morocco. The harvest of dry plant was carried out in February, and the harvest of the fresh plant in May. The plant material used in the extraction consists essentially of seeds and umbels of dry and fresh plant cut into pieces of about three centimeters.

Extraction protocol

The extraction of the plant's EO is carried out using a Clevenger device, a type of reactor 2000 ml flask. The total duration of the hydrodistillation is 3 h, with a temperature of 65°C, and distilled water volume of 500 ml. After calculating the performance, EO was stored at 4°C in the dark.

Chromatographic analysis of samples EO

For separation and determination of composition of the EO of *A. visnaga*, the analysis was performed at the Innovation Center (Fez-Morocco) through a coupling of Gas Chromatography-Mass Spectrum (GC-MS) type (Polaris Q) ion in electron impact hatch (AEs) with ionization energy of 70 ev. The column used is nonpolar capillary column type silica (WCOT Fused Silica) with a stationary phase (CP-SIL5CB), 50 m in length; the column temperature is programmed from 40 to 280°C at 3°C/min. The injector temperature is set at 240°C and the detector (ionization source) is 200°C. The flow rate of carrier gas (Helium) is set at 1 ml/min. The volume of sample injected is 1 µl of EO diluted in hexane. The components of the EO were identified by comparing their mass spectra with those listed in a type library (NIST-MS).

Antioxidant activity by the scavenging method of free radical 1,1-diphenyl-2-picrylhydrazyl(DPPH)

The evaluation of the antioxidant activity of *A. visnaga*, EO was made by the scavenging method of free radical DPPH. The absorbance measured at 517 nm is used to calculate the percent inhibition of DPPH radical which is proportional to the anti-radical power of EO of *A. visnaga*. DPPH molecule is characterized by a stable free radical with an absorption band in ethanol solution centered at about 517 nm, in presence of electron donor, DPPH is reduced to 1,1-diphenyl-2-hydrazine DPPH2 thus the violet color is changed to yellow due to the presence of picryl.

In order to evaluate the antioxidant potential through free radical scavenging by tested samples, the change in optical density of DPPH radicals is monitored. The solution of DPPH is prepared (0.0042 g in 200 ml of methanol). The sample extract of *A. visnaga* is prepared at a concentration of 1 mg/ml in methanol and is diluted with methanol and 2 ml of DPPH solution is added. After 30 min, the absorbance is measured at 517 nm.

Percent inhibition of DPPH

The percentage of the DPPH radical scavenging is calculated using the equation as given below:

$$\text{DPPH (\%)} = (\text{DO control})-(\text{DO spl})/(\text{DO control}) \times 100$$

Where, DO control: Optical density of the negative control tube. DO spl: Optical density of the sample.

IC_{50} determination

The IC_{50} value is the concentration which provides 50% of the activity of DPPH and that constitutes the antioxidant activity of EO determined graphically from the curve of the percentage inhibition versus concentration of EO [12]. This value is compared to that found in the reference antioxidant, ascorbic acid.

RESULTS AND DISCUSSION

Comparison of yield

The extraction from the dry plant gives a yield of 0.26% which is higher compared to that obtained from fresh plant (0.14%) and the yield of dry seeds is very low (0.02%) while oven dried umbels gave a yield of 0%.

If we compare the yields of our plant's EO with a plant produced in another region of Morocco, we note that the EO yield obtained for the studied plant of 0.26%, is relatively equal to that obtained for the same species in some regions of Morocco [7]; have superior performance [13] than plants from Tunis (0.175%) and is significantly lower than that of Constantine (1.3%) in Algeria [14] The studies reported in the literature [8,15,16] indicate that the performance of EOs vary according to the harvest period, method of extraction and drying of the plant.

Chemical composition

Sample 1 dry seeds: The chromatographic profile of EO from seeds of *A. visnaga* shows relative abundance of different compounds based on their output time in minutes. The first peaks appear after 12 min, the majority of components are grouped between 15 and 45 minutes with varying abundances.

This chromatogram identified 9 constituents with a 0.02% yield, where Menth-1-en-8-ol (24.33%), campholenal (13.6%), longipinene (5.53%) and longifolene (5.48%) predominate, other compounds represent the minority: Cymene (1.16%), carvacrol (4.37%), spathulenol (0.82%), himachalene (1.35%) and farnesylbromide (1.27%) (Tables 1-4; Figures 1-4).

Sample 2: Dried Ombelles

The chromatographic profile of EO from dry umbels has several compounds together between 15 and 45 min with a yield of 0.26%, in this case Menth-1-b-8-ol (25.57%), estragole (6.53 %) are major compounds, while cedrene (1.61%) and azulene (3.44%) are in minority (Table 2).

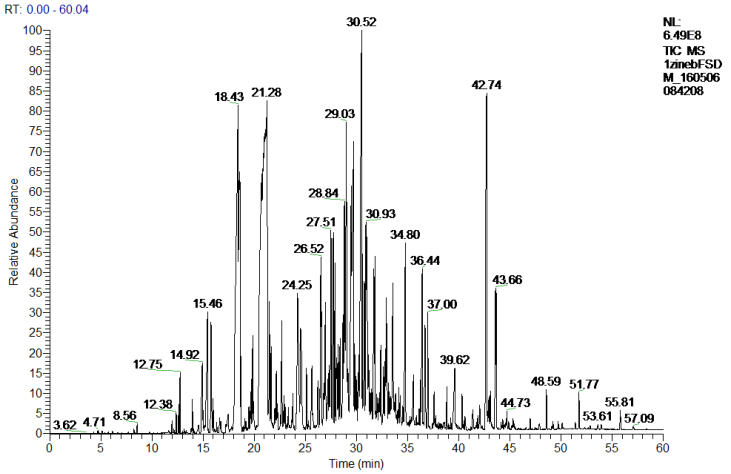

Figure 1: Chromatogram of EO dry seeds by GC/MS

Table 1: Chemical composition of dry seeds of the EO of *Ammi visnaga* from the region of Taounate (Morocco)

Pics	RT (min)	Area %	compounds
1	15.68	1.16	Cymene
2	19.79	13.6	Campholenal
3	21.30	24.33	Menth-1-en-8-ol
4	24.15	4.37	Carvacrol
5	27.49	5.48	Longifolene
6	28.84	5.53	Longipinene
7	30.71	0.82	Spathulenol
8	32.95	1.35	Himachalene
9	33.64	1.27	Farnesyl bromide

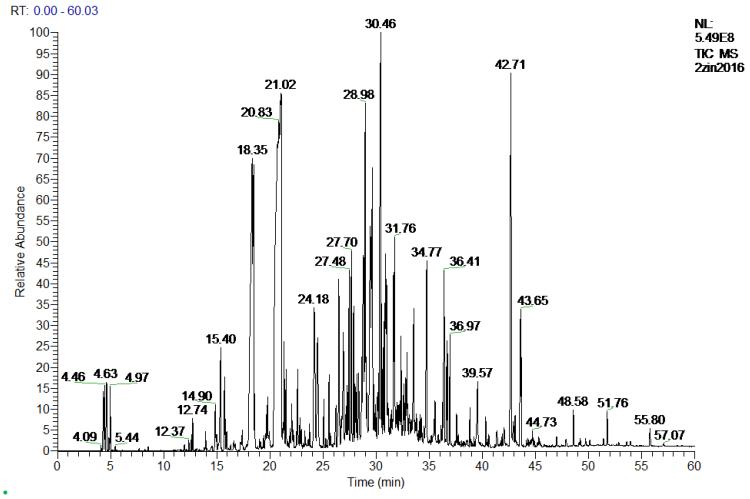

Figure 2: Chromatogram of EO dried umbels analyzed by GC/MS

Table 2: Chemical composition of dry umbels

Pics	RT (min)	Area %	Compounds
1	21.39	25.57	Menth-1-b-8-ol
2	27.68	1.61	Cedrene
3	28.79	6.53	Estragole
4	26.52	3.44	Azulene

The chromatographic analyzes of EO from fresh umbels highlighted the predominance of farnesyl bromide (3.14%), longifolene (3.14%), spathulenol (2.71%) and estragole (4.76%). While minority was composed of pulegone (1.16%), thymol (1.55%), thujopsene (1.95%), iso caryophyllene (1.42%), cedrene (2.14%), himachala-2,4diene (1.48%), guaine (1.48%), himachalenoxide (1.86%) and caryophyllene oxide (1.99%) (Table 3).

Table 3: Chemical composition of fresh umbels

Pics	RT (min)	Aire %	Compounds
1	22.70	1.16	Pulegone
2	24.24	1.55	Thymol
3	26.52	1.95	Thujopsene
4	27.51	1.42	Iso caryophyllène
5	27.73	2.14	Cedrene
6	27.92	1.42	Isoledene
7	28.04	2.14	Neoclovene
8	28.82	0.69	Cubenol
9	29.50	3.14	Farnesyl bromide
10	29.72	1.48	Himachala-2,4diene
11	29.82	3.14	Longifolene
12	30.51	1.48	Guaine
13	30.92	1.86	Himachal oxide
14	31.02	1.99	Caryophyllene oxide
15	31.70	2.71	Spathulenol
16	35.57	4.76	Estragole

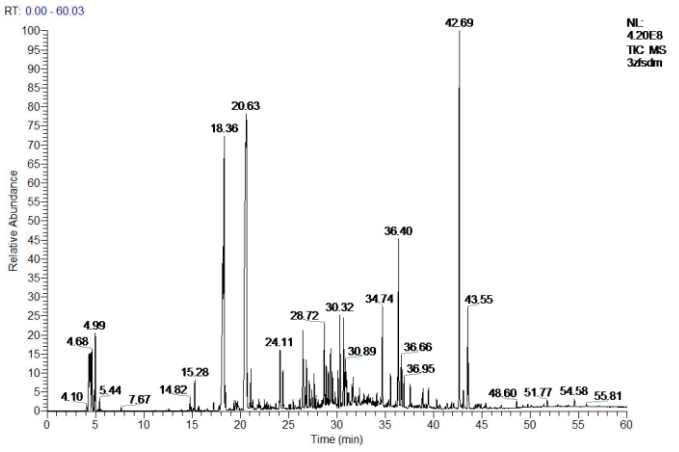

Figure 3: Chromatogram of EO fresh umbels analyzed by GC/MS

Different results were obtained by Satrani et al., [7] for the major constituents of *A. visnaga* of Morocco: amyl isobutyrate (about 16%), linalool (22.7%), 2-methyl butyrate isoamyl (approximately 27.7%) and valerate amyl (about 10%). They also found: α -terpinene (3.97%); α-thujene (1.37%), β-cymene (1.4%), limonene (1.95%), linalool oxide (cis: 2.1% trans: approximately 1.9%) and β-terpineol (1.12%) and for minority compounds: Monoterpenes (α-Thujene, α-pinene, α-terpinene), terpenes (β-Selinene, α-muurolene, α-farnesene), isobutyric esters isobutyrate, amyl acetates whose geranyl acetate, alcohols and phenols (β-Terpineol, borneol, linalool, α-terpineol). However campholenal (13.6%), longipinene (5.53%), longifolene (5.48%), estragole (6.53%), and Menth-1-b-8-ol (25.57%), the major constituents of our samples of EO were absent in the samples analyzed by the researchers. Furthermore Satrani et al., [7] have shown the presence of significant levels of methyl-2-isoamyl butyrate (approximately 27.7%) and relatively low levels of monoterpenes (α-thujene, α-pinene et α-terpinene), in its terpenes, isobutyric esters, alcohols and phenols on samples of *A. visnaga* from different regions of Morocco.

We lack in our samples some chemical elements of non-terpenic esters (43.3 to 49.1%), as monoterpene (38.5 to 39.1%), linalool (32%), isoamyl 2-methyl butyrate (24.2%) and isopentyl isovalerate (10%), which are considered the most abundant components of the EO of *A. visnaga* from Tunisia [13] and also isobutyl isobutyrate (14%), linalool (12.1%), dimethylbutanoic acid (30.1%), bornyl acetate (7.3%) and croweacin (12.2%) corresponding to the main constituents of Algerian *A. visnaga* EO [14] and only thymol was present in our sample of fresh *A. visnaga* umbels with 1.55%.

The chemical composition of *A. visnaga* EO varies between seeds and umbels, and from one region to another and according [8,15,16] the chemical composition of EOs varies also depending on the harvest period, and the drying mode of the plant. However, the same authors indicate that EO of *A. visnaga* of Moroccan origin is characterized by the presence of isobutyl amyl linalol, methyl-2-isoamyl butyrate with amyl valerate as a major component.

Antioxidant activity of EO *Ammi visnaga*

The linear curve of DPPH allows us to determine the IC_{50} value of *A. visnaga* EO [17]. IC_{50}EO = 56.053 ± 0.856 µg/ml. The results confirm the antioxidant activity of the EO of *A. visnaga* of Taounate region in Morocco. Indeed, the essence of *A. visnaga* and vitamin C could reduce the DPPH radical resulting in a change in the color of DPPH solution in methanol with values of 56.053 ± 0.856 µg/ml and 1.53 µg/ml, respectively. The absorbance of ascorbic acid (antioxidant standard) was measured under the same conditions as the samples. These results show that the EO of *A. visnaga* has antioxidant activity less effective than vitamin C.

Table 4: DPPH inhibition activity

dilution	Initial concentration (mg/ml)	Final concentration (mg/ml)	Optical density	Percent inhibition
1	1	0.058824	0.245	59.9018
2	0.5	0.029412	0.593	2.94599
4	0.25	0.014706	0.6	1.80033
8	0.125	0.007353	0.604	1.14566

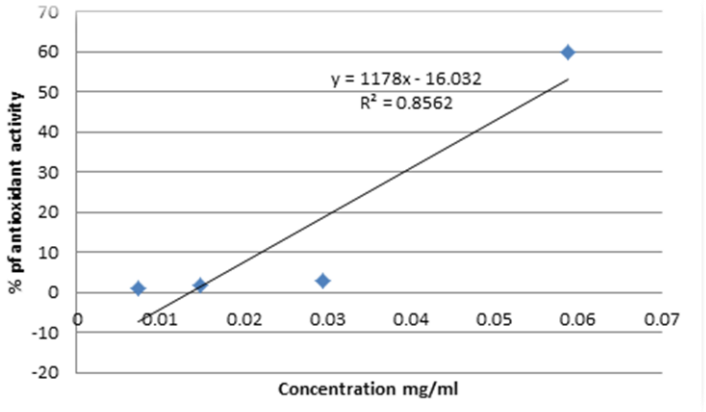

Figure 4: DPPH radical scavenging activity of *Ammi visnaga* essential oil

CONCLUSION

The present study was conducted to investigate the chemical composition and antioxidant activity of essential oil of *A. visnaga* from Morocco. The extraction of the EO by Clevenger gives a yield of 0.26% from the dry plant; which was more important than that obtained from the fresh plant (0.14%) and from dry seeds (0.02%). While oven dried plant gave a yield of 0%. The chromatograms of the EO of *A. visnaga* show that Campholenal (13.6%), longipinene (5.53%), longifolene (5.48%), estragole (6.53%), and the Menth-1-b-8-ol (25.57%), are the main constituents of the 29 identified. The chemical composition of the *A. visnaga* EO varies between seeds and umbels, and from one region to another and also depending on the time of harvest. The results obtained by the method of DPPH confirm the antioxidant potential of the essential oil of *A. visnaga* from Taounate region of Morocco. Indeed, the essence of *A. visnaga* and vitamin C could reduce the DPPH radical in solution of DPPH methanol with values of 56.053 ± 0.856 µg/ml and 1.53 µg/ml respectively, these results show that the essential oil of *A. visnaga* has antioxidant activity, but is less effective than vitamin C. The results of this study can contribute to the enhancement of EO of this plant by the local production of this species. The antioxidant activity also suggests application prospects in the fields of food, cosmetics, pharmaceutical and of herbal medicine. The results of this study could contribute to the valorization of this Moroccan aromatic and medicinal plant. This study can be considered as an important source of information on chemical properties and antioxidant power of the essential oil of Moroccan *A. visnaga L.*

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