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Total Polyphenol Content and Antioxidant Power of *Ammi visnaga* from Morocco

Ammor Kenza[1*], Bousta Dalila[2], Jennan Sanae[1], Chaqroune Abdellah[1], Mahjoubi Fatima[1]

[1]Laboratory of Materials and Process Engineering, University of Sidi Mohamed Ben Abdellah (USMBA) Fez, Morocco
[2]Laboratory of Neuroendocrinology and Nutritional Environment and Climate, University of Sidi Mohamed Ben Abdellah (USMBA) Fez, Morocco

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ABSTRACT

This study aims to investigate in vitro antioxidant power of hydro-ethanolic and aqueous extracts of aerial part of Ammi visnaga. Firstly, the extracts were analyzed for the phytochemical compounds contents. It was found that the amounts of tannins, flavonoids and coumarins are significant. However, the mucilage compounds were present in the aqueous extract, and not in the hydro-alcoholic extract. Furthermore, the results showed the presence of triterpenes and alkaloids only in the hydro-alcoholic extract. We also note the absence of saponins, cardiac glycosides, and holosides in both extracts.

The flavonoids and total phenols content were performed for both extracts. It shows higher content of flavonoids and total phenols in the hydro alcoholic extract.

Results obtained by all of the three radical scavenging activity methods used in this study (DPPH, FRAP and total antioxidant activity), showed higher antioxidant activity for the hydro-alcoholic extract than the aqueous extract of A. visnaga.

Keywords: *Ammi visnaga*, Extracts, Screening, Antioxidant activity, Polyphenol content

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INTRODUCTION

Morocco is characterized by a diversified climate and lithology, due to its geographical position and orographic context. This ecological diversity results in a great diversity of flora and a high rate of endemism [1]. Moroccan flora contains many plant species that have been used as medicinal and aromatic plants for centuries. These plants are used today in traditional medicine, food, pharmaceutical industry, perfumery and cosmetics [2].

Ammi visnaga belong to the family of umbellifereae which contained bioactive compounds, and known with regard to their economic importance.

A. visnaga is a wild plant indigenous to Egypt; it's distributed in North Africa, Europe, Eastern Mediterranean region, south western Asia, North America, Argentina, Chile, Mexico, and Atlantic Islands [3-5]. The dried fruits have been used for centuries in folk medicine for the treatment of skin disorder, psoriasis, as diuretic infusions and as therapy for kidney and bladder stones [6,7].

The khiline, visnagine and visnadine are the principal actives of the fruits of *A. visnaga*, they are used in the pharmaceutical industry [8]. The level of compounds in the dry fruits varies widely depending on genetic factors and environmental conditions [9].

Studies on the phytochemistry of *A. visnaga* have revealed the presence of diverse groups of chemical constituents such as pyrones, saponins and flavonoids [10].

The antioxidant activity of the butanolic extract of *A. visnaga* was determined by 2,2,mdiphényl-1-picryl-hydrazyl (DPPH) [11]. The free radical scavenging activity of the methanolic extract of Khella has been tested by DPPH radical method using Trolox as a reference standard by Jaradat [12].

The aim of this study is to investigate the antioxidant potential of hydro-alcoholic and aqueous extract of *Ammi visnaga* using three method based on the radical scavenging activity (DPPH free radical scavenging, FRAP and total antioxidant activity). Chemical screening was carried out to identify major biologically active phytochemical compounds.

MATERIALS AND METHODS

Plant material

The plant sample of *Ammi visnaga* constituted essentially of umbels was collected in July 2015 from Taounate (North Eastern Fes); the specimen identified was deposited in the herbarium of the laboratory of Biotechnology and preservation of natural resources (BPNR), Sidi Mohammed Ben Abdellah University, Fez, Morocco.

Preparation of the plant extract

Decoction

The extraction method was performed by taking 20 g of dried and pulverized umbels from *A. visnaga* with 250 ml of distilled water. The mixture was heated (reflux system) for 30 min. The mixture was filtered using Whatman filter paper and concentrated under reduced pressure.

Soxhlet extraction

In a Soxhlet system, 50 g of dried plant powder was put in a cellulose cartridge and extracted with 250 ml of ethanol-water (70/30). The extraction process continues still the solvent in siphon tube of an extractor become colorless. After that the extract was filtered and been concentrated under reduced pressure.

Qualitative phytochemical analysis

Phytochemical Screening of dry extracts was achieved through simple methods [13-16].

Determination of the total phenolic content

The total phenolic content of the extracts was determined by the method of Folin-Ciocalteu [17]. A quantity of 20 µl of the extract was mixed with 1.16 ml of distilled water, 100 µl of Folin-Ciocalteu reagent and 300 µl of sodium carbonate (Na_2CO_3) to 20%. The whole is incubated at 40°C for 30 min and the reading is taken against a white using a spectrophotometer at 760 nm.
The absorbance was measured with a white made from distilled water using a spectrophotometer UV-Visible kind Selecta. A calibration curve for various concentrations of Gallic acid was plotted. The data are presented as the average of triplicate analyses.

Determination of flavonoid contents

The flavonoids assay was performed according to the method described by Saeedeh and Asna [18]. A quantity of 0.5 ml of the extract was mixed with 0.5 ml of aluminum chloride ($AlCl_3$). After 1 h incubation at room temperature, the absorbance is measured at 420 nm. Quantification of flavonoids is determined based on a linear calibration curve produced by the Quercetin at different concentrations and under the same conditions as the sample. The results are expressed in microgram equivalents of Quercetin per milligram extract (QE mg/g of extract). The data are presented as the average of triplicate analyses.

Evaluation of the Antioxidant activity

The DPPH method

The free radical-scavenging activity of extracts was evaluated by 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) according to the method reported by Quettier-Deleu et al. [19]. 50 µL of different concentrations of the extracts was mixed with 2 ml of a 60 µM DPPH methanolic solution. The mixture was shaken vigorously and incubated for 20 min in the dark at 25◦C. The absorbance of the samples was measured spectrophotometrically at 517 nm. Butylated hydroxytoluène (BHT) was used as positive control. The antiradical capacity of the studied essential oil was calculated using the following formula:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1)]/A_0 \times 100$$

Where: A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.
The antiradical activity was expressed as IC_{50}, which is the antiradical concentration required to cause 50% of inhibition. A lower IC_{50} value corresponds to a higher antioxidant capacity of the extract. The IC_{50} was calculated by plotting inhibition percentages against concentrations of the sample. The experiment was repeated three times and the results were expressed as mean ± SD.

Ferric-reducing Antioxidant power (FRAP)

The reductive potential has been examined by the transformation of Fe^{+3} to Fe^{+2} in the presence of an antioxidant this test is realized according to the procedure of Oyaizu [20]. In a test tube containing 1 ml of sample solution, 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide [$K_3Fe(CN)_6$] (1%) was added. The mixture was incubated at 50°C for 20 min. After incubation, 2.5 ml of trichloroacetic acid 10% was added to the mixture and centrifuged at 3000 rpm for 10 min. Then, 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1%) and the absorbance was measured spectrophotometrically at 700 nm.

Evaluation of the total antioxidant capacity by Phosphomolybdenum method

Total antioxidant capacity (TAC) of the extracts was evaluated by Phosphomolybdenum method Prieto [21]. This technique is based on the reduction of molybdenum Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/ Mo (V) complex at acid pH. A volume of 0.3 ml of the extract was mixed with 3 ml of the reagent solution (0.6 M sulfuric acid, 28 mM sodium and 4 mM of ammonium molybate). The tubes were incubated at 95°C for 90 min. After cooling, the absorbance of the solutions is measured at 695 nm against the blank which contains 3 ml of the reagent solution and 0.3 ml of methanol and which was incubated under the same conditions as the sample. Total antioxidant capacity is expressed in milligrams of ascorbic acid per gram of dry matter (mg EAA/g MS). The experiments are repeated three times.

RESULTS AND DISCUSSION

Extraction yield

The extraction results of the *Ammi visnaga* aerial parts, showed a higher yield for the hydro-alcoholic extract with a value of 23.40% followed by the aqueous extract with a yield of 18.60%.

Phytochemical screening

The results indicated in Table 1 of the phytochemical tests carried out on the aqueous hydro-alcoholic extracts of the aerial parts of *A. visnaga* show the presence of gallic tannins, flavonoids and coumarins in both extracts. The mucilages are present only in the aqueous extract of the plant. Alkaloids, Triterperne and sterols, heterosides triterpenes are present only in the hydroalcoholic extract. On the other hand the Cardiac Glicosides, oses and holosides are absent in both extracts.

Table 1: Phytochemical screening of the aqueous and the hydro-alcoholic extracts of *Ammi visnaga*

Chemical constituent			Hydro alcoholic Extract	aqueous Extract
Polyphénols	Tanins	Tanins totaux	+	+
		Gallic tanins	+	+
		catechin tannins	-	-
	Flavonoides	Flavonols	+	+
		Anthocyanes	-	-
		Leucoanthocyanes	-	-
Alcaloides			+	-
triterpènes et sterol			+	-
Mucilage			-	+
Saponosides			-	-
Hétéroside stéroidiques			-	-
Triterpènes hétérosides			+	-
Coumaines			+	+
Glycosides cardiaque			-	-
Oses et holosides			-	-

+: Présence; -: Absence

Total phenolic and flavonoid contents

The total phenol content was determined in comparison with a standard which is Gallic acid. The results expressed in terms of mg GA/g of extract. The hydro-alcoholic extract has higher phenol content than the aqueous extract.

$$Y=1.575X-0.0229 \quad (R^2=0.959)$$

The total flavonoid content was determined in comparison with Quercetin. The hydro-alcoholic extract has higher flavonoid content than that of the aqueous extract.

$$Y=0.0489x-0.0416 \quad (R^2=0.977)$$

Table 2: Total phenolic and flavonoids contents in the aqueous and hydro-alcoholic extracts of *Ammi visnaga*

Extracts	Total phenolique content (mg GAE/g extract)	Flavonoïde content (mg QE/g extract)
Ammi visnaga Hydro alcoholic Extract	66.94 ± 3.10	7.72 ± 0.082
Ammi visnaga aqueous Extract	66.10 ± 1.27	5.21 ± 0.064

Antioxidant activity

Determination of free radical scavenging activity by DPPH method

The anti-free radical capacity of the hydro-alcoholic and aqueous extracts of *A. visnaga* was tested by the DPPH method using BHT as a reference standard. The concentration varies between 0.01 and 6 mg/ml.

Inhibition zero was considered for the solution that contained only DPPH without plant extract. The result showed strong antioxidant activity for the two extracts with an IC_{50}=3.19 mg/ml for the hydro-alcoholic extract and an IC_{50}=3.5 mg/ml for the aqueous extract comparable to BHT the standard which has an IC_{50}=0.17 mg/ml, the detailed results are presented in Tables 2 and 3.

Table 3: Inhibition activity of BHT and Different extracts of *Ammi visnaga*

Plant	DPPH IC_{50} (mg/ml)
Ammi visnaga Hydro-alcoholic extract	3.19
Ammi visnaga aqueous extract	3.5
BHT	0.17

Reducing power by FRAP method

Potential measures reduce the ability of a sample to act as an electron donor and, therefore, reacts with a radical connection by converting it to more stable products and thus ends radical chain reactions.

Figure 1 shows the reducing powers of the aqueous extracts and hydro-alcoholic extracts of *A. visnaga* and the BHT standard. The power of reduction of the extracts increases with the concentration. The hydro-alcoholic extract showed stronger the reduction of power compared to the aqueous extract.

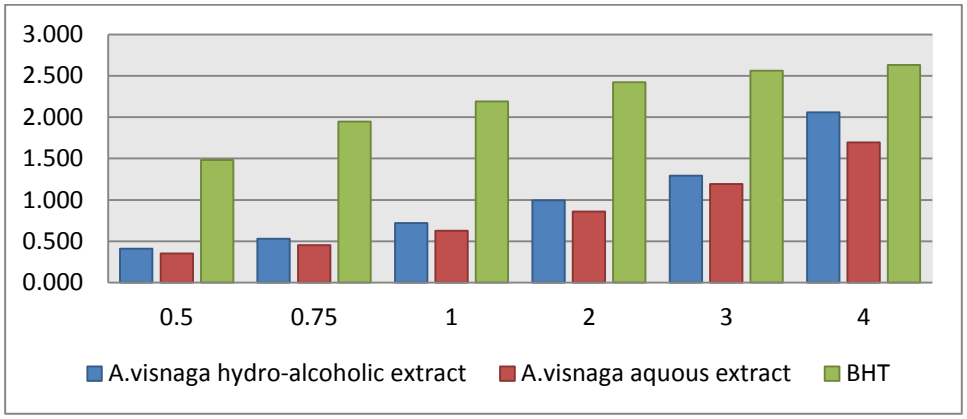

Figure 1: Reducing power of different *Ammi visnaga* extract compared to BHT standard

Evaluation of the total Antioxidant capacity by Phosphomolybdenum method

The total antioxidant activity of the aqueous and hydro-alcoholic extracts is expressed in equivalents of BHT (Table 4). The test is based on the reduction of Mo (VI) to Mo (V) by the extract and the subsequent formation of a phosphate of a complex green/Mo (V) complex at an acidic pH. The method is quantitative phosphomolybdenum since the antioxidant activity is expressed in the number of equivalents of BHT [21]. The hydro-alcoholic extract has a capacity greater than that of the aqueous extract.

Table 4: total antioxidant activity of the extracs *Ammi visnaga* (n=3)

	TFC (mg BHT/g of extract)
Hydro alcoholic extract	72.79 ± 7.28
aqueous extract	42.53 ± 4.25

DISCUSSION

In the present study, the antioxidant capacity of *Ammi visnaga* extracts was evaluated by using three different methods. DPPH, phosphomolybdenum and reducing power are antioxidant tests commonly used for the characterization of plant extracts. In fact, the results of a single dosage may give a reducing suggestion of the antioxidant properties of the extracts. In addition, the chemical complexity of the extracts, is often a mixture of several compounds with differences in functional groups, polarity and chemical behavior, could lead to scattered results, depending on the test used. Therefore, an approach with multiple trials is strongly recommended [22].

Concerning our study the phytochemical screening of the aerial parts of *A. visnaga* was realized on the aqueous and hydro-alcoholic extracts show the presence of gallic tannins, flavonoids and coumarins in both extracts. The mucilages are present only in the aqueous extract of the plant. Alkaloids, Triterperne and sterols, heterosides triterpenes are present only in the hydroalcoholic extract. On the other hand the Cardiac Glicosides, oses and holosides are absent in both extracts. For Jaradat [12] the organic extractions was performed by Soxhlet using different solvent (methanolic, acetone and ethanolic) and the aqueous extract was realized by decoction, The phytochemical characteristics of *Ammi visnaga* shows that proteins, phenols, tannins, falvonoids, glycosides and steroids were present in all plant extract. In the other hand carbohydrates, terpenoids and saponins were absent [12]. The phytochemical study of essential oils of *A. visnaga* revealed the presence of therapeutic molecules such as polyphenols and alkaloids, Tannins, Flavonoids, Mucilages Sterols and Triterpenes on the other hand the absence of Saponins [23].

These bioactive molecules highlighted also contribute to the virtues of the plant, considering in particular the benefits of polyphenols with respect to cardiovascular diseases [24] and the role of tannins and mucilags in the management of diabetes mellitus by their capacity to delay the release of glucose and their antioxidant effects [25,26].

The *A. visnaga*'s hydro-alcoholic extract have higher values of polyphenols content than the aqueous extract we found higher values than those found by Wadhwa [27].

In our study and based on the three assays of the antioxidant activity of the *A. visnaga* extracts, the hydro-alcoholic extract exhibited a higher antioxidant activity than the aqueous extract.

According to the results reported by Bencheraiet et al., the aerial parts of *A. visnaga* had higher ability for scavenging DPPH radicals (8.77 µg/ml) than the standard (3.01 µg/ml), which may partly be explained by the different types of solvents used for the extraction and, therefore, different types of compounds isolated [11]. The free radical scavenging activity of the methanolic extract of *A.visnaga* has been tested by DPPH radical method using Trolox as a reference standard. The result revealed a high antioxidant activity with IC_{50}=6 µg/ml which comparable to Trolox stanrd which has an IC_{50} of 1.5 µg/ml [12].

The DPPH scavenging ability of *A. visnaga*'s essential oils and reference BHT was realized by Miguel [28]. The two essential oils studied reduced the concentration of DPPH free radical with lower efficiency than that of the positive control. The reason of this poor activity, is due probably to its lack or low amount of phenolic contents; synergistic or antagonistic effect of its components. Thus, the lower efficacy of *A. visnaga* oils could be explained by their very poor amount of aromatic components.
Depending on Keddad [29] which have extracted phenols from the plant, the extract have an important antioxidant activity (0.129 mg/ml) compared to the standard (0.089 mg/ml).

It is well known that phenolic plant compounds provide a better result in the presence of free radicals and antioxidants. The results obtained in our analyses show that the content of phenolic compounds estimated in *A. visnaga* extracts are probably responsible for the antioxidant activity.

CONCLUSION

In conclusion, the results of this study have shown that hydro-alcoholic extract of *Ammi visnaga* is rich in total phenols and flavonoids and provide stronger scavenging effect tested by three methods (DPPH free radical scavenging, FRAP and total antioxidant activity).
This antioxidant effect could be explained by the richness of *A. visnaga* hydro-alcoholic extract on tannins, total phenols and flavonoids compounds.

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