

Phytochemical Screening, Antimicrobial and Antioxidant Activity of *Ammi visnaga* L. Extracts

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ABSTRACT

Ammi visnaga L. extracts were examined for the presence of phytochemicals, antimicrobial activities, and scavenging potentials. The aerial part of this plant underwent warm extraction using three different solvents: hexane, dichloromethane and ethanol. The phytochemical analysis revealed the presence of polyphenols, tannins, flavonoids, glycosides, and reducing sugars in the ethanolic extract. In the dichloromethane extract, polyphenols and glycosides were identified, while in the hexane extract, polyphenols, flavonoids, and glycosides were detected. Antimicrobial activity was determined using disc diffusion method. Results indicate that the dichloromethane extract exhibited the largest zone of inhibition, measuring 10 mm against *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) was recorded as 10 μ L/mL, while the minimum bactericidal concentration (MBC) was below 10 μ L/mL. However, no antimicrobial activity was observed against *Klebsiella pneumoniae* and *Acinetobacter baumannii*. Additionally, antioxidant activity was examined using the DPPH (2,2-diphenyl-1-picrylhydrazyle) assay. The ethanolic extract demonstrated the highest antioxidant power with an IC₅₀ value of 0.843 ± 0.199 mg/mL against 0.095 ± 0.009 mg/mL of ascorbic acid which is used as a reference.

Keywords: *Ammi visnaga* L., antibacterial activity, antioxidant activity, phytochemical analysis.

INTRODUCTION

Aromatic and medicinal plants (AMP) have a long history of use in traditional medicine for treating various ailments [Moalim et al. 2018; Khalil et al. 2020]. Essential oils and various plant extracts from these sources have garnered significant interest as natural products [Tepe et al. 2004]. There is growing attention in investigating these natural materials as potential sources for new antibacterial agents [Bonjar et al. 2003; Betoni et al. 2006] and antioxidants [Karkouri et al. 2020]. However, despite their potential, aromatic and medicinal plants (AMPs) are presently underutilized in the medical, pharmaceutical and agri-food areas [Karkouri et al. 2020]. *Ammi visnaga* L., a commonly known

annual herbaceous plant referred to as Khella, Visnaga, and Toothpick weed, is among the aromatic and medicinal herbs categorized within the Apiaceae (Umbelliferae) family. Khella, originally native to the Mediterranean region [Talaat et al. 2014], has propagated and extended its presence across various parts of the world. *Ammi visnaga* L. can be found in various regions including North and South America (the Atlantic islands, Argentina, Mexico, and Chile), Iraq, Iran, and Asia [Khalil et al. 2020]. Despite extensive exploration into the antioxidant capacity of the Apiaceae family, the diverse therapeutic effects of *Ammi visnaga* L. have only recently gained attention in research. Limited studies have specifically focused on the antioxidants present in *Ammi visnaga* L. [Kamal et al. 2022]. In the

agricultural field, khella is also employed as both a herbicide and bactericide to protect plants [Khalil et al. 2020; Iacobellis et al. 2005]. *Ammi visnaga* L. has a well-established historical use in the medical field for the treating various diseases, including coronary heart disease [Khadhri et al. 2011], nephritic disease [Vanachayangkul et al. 2010; Alam et al. 2018], angina pectoris, as well as for its antispasmodic, bronchial, and vasodilator asthma [Gunnaydin and Beyazit 2004; Nada et al. 2014]. Khella exhibits antioxidant, antibacterial, antifungal, and anticancer properties [Kamal et al. 2022; Kooti et al. 2017]. Moreover, khellin extracted from *Ammi visnaga* L. has demonstrated the ability to notably elevate the levels of high density lipoprotein cholesterol (HDL) while maintaining unchanged concentrations of total cholesterol or triglycerides [Harvengt et Desager 1983].

In the present study, *Ammi visnaga* L. extracts were tested for the presence of phytochemicals and assessed for their antibacterial activities against six bacterial strains: *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Enterobacter cloacae* and *Acinetobacter baumannii*. The antibacterial evaluation was conducted using the agar diffusion method. Additionally, antioxidant activities were determined using the DPPH radical inhibition method.

MATERIALS AND METHODS

Preparation of *Ammi visnaga* L. extract

Ammi visnaga L. was harvested from Sidi kadem region of Morocco in July 2017. The plant materials (the aerial part) were ground after being cleaned, dried in the shade, and at room temperature (28 °C) for 13 days. The extraction was performed using the Soxhlet [Oubihi et al. 2020]. Thirty grams of the dried plant powder were extracted in 300 mL of hexane for three hours. Subsequently, the extraction residue was subjected to extraction in dichloromethane for five hours and then in absolute ethanol for seven hours. The resulting extracts were recuperated after concentration by evaporation in vacuo, and kept for their future use at 4 °C in a refrigerator.

Phytochemical screening

Phytochemical screening was performed using standard procedure. The alkaloids were

determined using the Dragendorff's test, while steroids and triterpenes were determined using the Liebermann-Burchard test [Iqbal et al. 2015]. Polyphenols were detected through their reaction with ferric chloride, while flavonoids were determined by their reaction to cyanidin and tannins using the Stiasny's test [N'Guessen et al. 2009]. The reducing sugars were determined according to the Fehling's test [Trease and Evans 1996]. The saponins were determined using the method described by Adegoke et al. [2010]. The glycosides were detected using the Keller Kiliani test [Ayoola et al. 2008]. The coumarins were determined using NaOH as the reagent [Ayoola et al. 2008].

Antimicrobial activity

Diffusion method on a solid medium

Antibacterial activity was performed using the Muller Hinton solid medium diffusion method [Mgamat et al. 2023]. The bacterial strains used are *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Enterobacter cloacae* and *Acinetobacter baumannii*. They were chosen for their pathogenicity and their ability to contaminate food. On a Petri dish containing an agar medium, a microbial suspension with an optical density of 1 McFarland is dispersed. *Ammi visnaga* L. extracts were dissolved in DMSO (Dimethylsulfoxide). Whatman absorbent paper discs with a diameter of 6 mm, sterilized by autoclave (121 °C for 20 minutes) then soaked in the test extract. The prepared discs are placed on the surface of the inoculated agar, the whole is incubated at 37 °C for 24 hours. The inhibition zone diameter was measured in millimeters. Penicillin (5 µg), gentamicin (500 µg) and oxacillin (5 µg) are the antibiotics used as positive controls. Each test was repeated three times. The determination of the minimum inhibitory concentration (MIC) for *A. visnaga* extracts was carried out following the method described by Hajib et al. [2020].

Antioxidant activity

The antioxidant activity of each *Ammi visnaga* L. extract was determined using the DPPH· (1,1-diphenyl-2-picryl hydrazyl) radical reduction method as described by Haida et al. [2020] with some modifications. A solution of DPPH was prepared in ethanol at a concentration of 76

μM (0.03 mg/mL). Subsequently, 2 mL of this solution was added to 0.1 mL of the extract at various concentrations. Simultaneously, a blank containing methanol and the DPPH solution was prepared. The mixture was agitated and left at room temperature in the shade for 30 minutes. Absorbance was measured at 517 nm using a spectrophotometer. Ascorbic acid was used as a reference antioxidant and tested at equivalent concentrations. The test was repeated three times. The inhibition percentage of the DPPH \cdot radical can be calculated using the following Equation 1:

$$(\%) \text{ inhibition} = [(Ab - Ae)/Ab] \times 100 \quad (1)$$

where: Ab – absorbance of the blank, Ae – absorbance of the sample.

To determine the sample concentration required to inhibit 50% of the DPPH radicals, the IC_{50} inhibitory concentration (2) is utilized. The linear section of the regression curve indicates the variation in the percentage inhibition according to the different concentrations for each tested sample.

$$IC_{50} = (50 - b)/a \quad (2)$$

where: a – slope of the line, b – intercept of the line.

RESULTS AND DISCUSSION

Phytochemicals screening

The qualitative phytochemical analysis results for the hexanic, dichloromethane and ethanolic extracts of *Ammi visnaga* L. are presented

in Table 1. Consistently, polyphenols and glycosides were present in each extract. Furthermore, alkaloids and saponins were absent in all three extracts. Tannins (catechic and gallic) and reducing sugars were specifically identified in the ethanolic extract and were not detected in other the extracts. However, concerning other secondary metabolites like flavonoids and coumarins, the presence varied among the extracts. These results in accordance with those of Amine et al. [2015] which similarly indicated the absence of alkaloids and saponins in ethanolic extract. On the contrary, the results of Moalim et al. [2018] have demonstrated the presence of alkaloids and saponins specifically in the hexanic and ethanolic extract of *Ammi visnaga* L. Conversely, Aourabi et al. [2018], identified the presence of coumarins and steroids in the ethanolic extract of the same plant. These contradictory results among various studies highlight the variability in phytochemical compositions obtained from different extraction methods or plant sources, suggesting the complexity of the plant's chemical profile and the importance of considering diverse extraction techniques in phytochemical analysis.

Antimicrobial activity

The antibacterial activity of *Ammi visnaga* L. extracts against the tested microorganisms was evaluated quantitatively and qualitatively by measuring the inhibition zones, determining both the minimum inhibitory concentration (MIC), and the minimum bactericidal concentration (MBC). The results have been presented and organized in Tables 2 and 3 and Figure 1. Based

Table 1. Phytochemicals screening of *Ammi visnaga* L. extracts

Tests		<i>A. visnaga</i> extract		
		HE	DE	EE
Polyphenols		+	++	+++
Alkaloids		-	-	-
Tannins	Catechic	-	-	+
	Gallic	-	-	+
Flavonoids		+	-	+
Steroids and triterpenes		-	-	-
Reducing sugars		-	-	+
Saponins		-	-	-
Glycosides		+	+	+
Coumarins		+	+	-

Note: HE – hexanic extract, DE – dichloromethane extract; EE – ethanolic extract.

on these findings, it is evident that the tested extracts exhibited distinct antibacterial activities. Specifically, the hexanic extract demonstrated inhibition of growth in a single strain, *Enterobacter cloacae*, with an inhibition zone diameter of 8 mm. Similarly, the ethanolic extract displayed inhibition solely against *Staphylococcus aureus*, with an inhibition zone diameter of 7 mm. On the other hand, the dichloromethane extract showed antibacterial effects against *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Escherichia coli* with varying inhibition zone diameters ranging from 7 mm to 10 mm. For *Klebsiella pneumoniae* and *Acinetobacter baumannii*, the tested extracts did not exhibit any sensitivity. The observed inhibitory activity was notably lower when compared to gentamicin, which demonstrated larger inhibition zone diameters of up to 37 mm. However, it's worth noting that *Escherichia coli* displayed no sensitivity to gentamicin. On the other hand, all tested strains showed no sensitivity to penicillin and oxacillin (Table 2).

The differences observed in the antimicrobial activities among the studied extracts may be attributed to variations in microbial sensitivity to various chemical components [Ghareeb et al. 2011]. The evaluation method can have significantly influence antibacterial activity results. For instance, compared to the agar diffusion method, the well diffusion method on agar has proven more effective in evaluating the antibacterial properties of organic and aqueous extracts of *Euphorbia fusiformis*, *Rhus coriaria* and *Zataria multiflora* [Natarajan et al. 2005; Fazli et al. 2007; Athamena et al. 2010]. Previous studies on *Ammi visnaga* L. indicate its essential oil, at doses of

1/250 (V/V) and 1/500 (V/V), exhibited inhibitory activity against *Staphylococcus aureus* and *Escherichia coli* strains, respectively [Satrani et al. 2004]. Additionally, studies on *Ammi visnaga* L. seeds highlighted the inhibition effects against *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* strains, satisfactory results were achieved for the ethanolic extract with an inhibition zone diameters of 21, 24, and 20 mm, respectively [Moalim et al. 2018].

The results presented in Table 3 indicate that strains such as *Enterobacter cloacae*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli* required a MIC of 10 μ L/mL to inhibit growth, while the MBC was found to be less than 10 μ L/mL for all the tested extracts. In a similar study conducted by Ghareeb et al. [2011] on *Ammi visnaga* L., it was reported the ethanolic extract inhibited the growth of *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* strains, with MICs of 12.5 mg/mL for *Escherichia coli* and *Klebsiella pneumoniae* and 50 mg/mL for *Staphylococcus aureus*. Additionally, Onder et al. [2019] found that *Ammi visnaga* L. hexane extract from Turkey inhibited the growth of *Klebsiella pneumoniae* and *Staphylococcus aureus* with MICs of 2.5 mL/mL and 10 mg/mL, respectively, while exhibiting no inhibition effect on the *Escherichia coli* strain. However, a separate study performed in Palestine by Amin et al. [2015] revealed that the *Ammi visnaga* L. organic extract exhibited significant antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Acinetobacter baumannii* strains, with MIC values of 0.35, 2.78, and 5.56 mg/mL, respectively.

Table 2. The antibacterial activity of the *Ammi visnaga* L. extracts by the method of diffusion on a solid medium

Microorganisms	Inhibition zone diameter (mm)*					
	Extract			Antibiotic		
	HE	DE	EE	P	Ox	Gn
Gram-positive <i>Staphylococcus epidermidis</i>	NA	7±0.1	NA	NA	NA	37 ± 1.02
<i>Staphylococcus aureus</i>	NA	10±1.06	7±0.3	NA	NA	30 ± 0.5
Gram-negative						
<i>Klebsiella pneumoniae</i>	NA	NA	NA	NA	NA	7 ± 0.21
<i>Enterobacter cloacae</i>	8 ± 0.02	NA	NA	NA	NA	23 ± 1.15
<i>Escherichia coli</i>	NA	7±1.2	NA	NA	NA	NA
<i>Acinetobacter baumannii</i>	NA	NA	NA	NA	NA	21 ± 1.3

Note: * Diameter of the inhibition zone including the disc diameter of 6 mm, by the agar disc diffusion method, HE – hexane extract; DE – dichloromethane extract; EE – ethanol extract; P – Penicillin (5 μ g), Gn – gentamicin (500 μ g), Ox – oxacillin (5 μ g), NA – non active.

Table 3. Minimum inhibitory concentration (MIC) and bactericidal (MBC).

Microorganisms	Extracts					
	MIC ($\mu\text{L/mL}$)			MBC ($\mu\text{L/mL}$)		
	DE	HE	EE	DE	HE	EE
Gram-positive						
<i>Staphylococcus aureus</i>	10 $\mu\text{L/mL}$	NT	10 $\mu\text{L/mL}$	<10 $\mu\text{L/mL}$	NT	<10 $\mu\text{L/mL}$
<i>Staphylococcus epidermidis</i>	10 $\mu\text{L/mL}$	NT	NT	<10 $\mu\text{L/mL}$	NT	NT
Gram-negative						
<i>Enterobacter cloacae</i>	NT	10 $\mu\text{L/mL}$	NT	NT	<10 $\mu\text{L/mL}$	NT
<i>Escherichia coli</i>	10 $\mu\text{L/mL}$	NT	NT	< 10 $\mu\text{L/mL}$	NT	NT

Note: NT – not tested.

Antioxidant activity

Percentage of inhibition

The Figure 1 illustrates the percentage of DPPH radical inhibition in relation to the concentration of tested extracts and ascorbic acid. The antioxidant power results of the *Ammi visnaga* L. extracts showed that the ethanolic extract displayed strong inhibition exceeds 66% at concentrations around 1.4 mg/mL. In contrast, the dichloromethane and hexanic extracts exhibited lower inhibition percentages, measuring less than 40% and 20% respectively, at concentrations near 14 mg/mL. The percentage of inhibition for all extracts remained lower than the ascorbic acid.

Determination of IC50

The IC50 is used to determine the extracts antioxidant power. This is the concentration of extract required to scavenging 50% of the DPPH

radical. Table 4 illustrates the IC50 values for the *Ammi visnaga* L. extracts and ascorbic acid, which are indicative of their antioxidant potency. Notably, the ethanolic extract displayed significant antioxidant activity ($\text{IC}_{50} = 0.843 \pm 0.199$ mg/mL), albeit lower than that of ascorbic acid ($\text{IC}_{50} = 0.095 \pm 0.009$ mg/mL). Conversely, the dichloromethane and hexane extracts exhibited relatively lower antioxidant activities, with IC50 values of 14.663 ± 4.470 mg/mL and 42.672 ± 3.354 mg/mL respectively. In a study by Bencheraiet et al. [2011], the butanol extract of *Ammi visnaga* L. demonstrated strong antioxidant activity with an IC50 of 8.77 $\mu\text{g/mL}$. Additionally, previous studies conducted in Sudan showed the substantial reduction capacities of the ethanolic and aqueous extracts of *Ammi visnaga* L., reporting IC50 values of 41 $\mu\text{g/mL}$ and 47 $\mu\text{g/mL}$, respectively [Hilmi et al. 2014]. Karkouri et al. [2020], revealed that the ethyl acetate fraction of hydracetic and hydromethanolic extracts

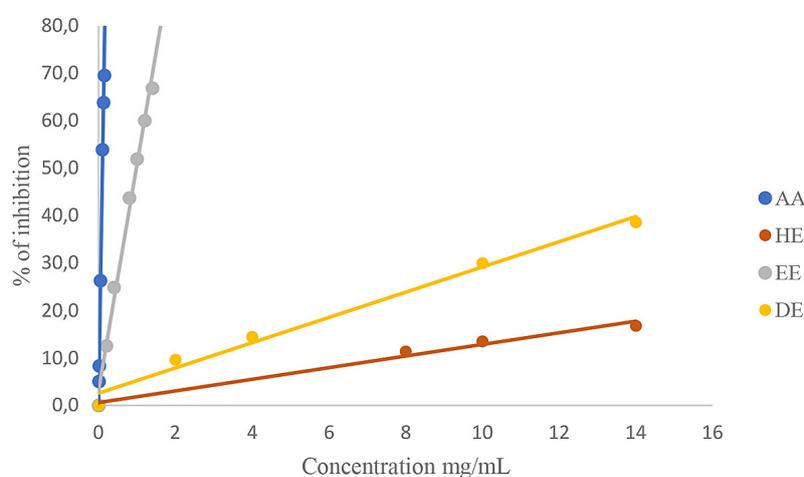


Figure 1. Percentage of inhibition for the *Ammi visnaga* L. extract and ascorbic acid, AA – ascorbic acid; HE – hexane extract; DE – dichloromethane extract, EE – ethanolic extract

Table 4. Antioxidant power of *Ammi visnaga* L. extracts and ascorbic acid, expressed by the IC₅₀ value obtained in the DPPH test

Antioxidants	DPPH IC ₅₀ (mg/mL)
Ascorbic acid	0.095 ± 0.009
HE	42.672 ± 3.354
DE	14.663 ± 4.470
EE	0.843 ± 0.199

of *Ammi visnaga* L. revealed strongest anti-free radical activity, displaying IC₅₀ values of 12.87 µg/mL and 10.88 µg/mL, respectively.

CONCLUSION

In conclusion, the phytochemical analysis of the ethanolic extract of *Ammi visnaga* L. reveal the presence of polyphenols, tannins, flavonoids, glycosides and reducing sugars. While for dichloromethane and hexane extracts only contain polyphenols and glycosides, and coumarins. The study of the antioxidant activity of *Ammi visnaga* L. extracts using the DPPH free radical reduction method showed that the ethanol extract exhibits strong antioxidant activity. This effect could be attributed to its richness in phenolic compounds. Among the tested microbial strains, varied sensitivity to *Ammi visnaga* L. extracts was observed, except for *Klebsiella pneumoniae* and *Acinetobacter baumannii* which showed resistance to the tested extracts. Further investigations are warranted to identify the specific chemical components responsible for the extract's antioxidant and antimicrobial properties.

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