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RESEARCH ARTICLE

# Antibacterial activity and chemical composition of the essential oil of *Ammi visnaga* L. (Apiaceae) from Constantine, Algeria

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**Abstract**: The essential oil of fresh aerial part of *Ammi visnaga* L. (Apiaceae), obtained by hydrodistillation, in a Clevenger-type apparatus, was GC/MS analyzed. Twenty one compounds were characterized representing 97.3% of the essential with isobutyl isobutyrate (14.0 %), linalool (12.1%), 2,2-dimethylbutanoic acid (30.1%), thymol (6.0%), bornyl acetate (7.3%) and croweacin (12.2%) as the major components. The essential oil exhibited the best antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* strains.

**Keywords**: *Ammi visnaga* L.; Apiaceae; essential oil; antibacterial.

## Introduction

*Ammi visnaga* L. (Apiaceae) is a perennial herb widely distributed in the Mediterranean area. The genus *Ammi* comprises 3 species in the Algerian flora (Quezel and Santa 1963). Furanochromones (khellin and visnagin) and flavonoids have been reported from *Ammi visnaga* L. (Bencheraiet et al. 2010, Cisowski 1986; Franchis et al. 1985; Saleh 1983) but the essential oil of the species growing in Morocco has been reported to contain linalool and aliphatic esters as the main components (Lamiri et al. 2001). This oil is locally used to treat asthma. In continuation of our works on Apiaceae essential oils studies (Boudiar et al. 2011 ; Boutaghane et al. 2004 ; Chibani et al. 2011; Daroui et al. 2010 ; Labed et al. 2011 ; Vérité et al. 2004) we report here, the chemical composition and the antibacterial activity of *Ammi visnaga* L. collected at Constantine (Algerian eastern).

## Material and methods

### *Plant material*

*Ammi visnaga* L. was collected in April 2009 from Didouche Mourad-Constantine (North Eastern Algeria) and identified by Pr. Gérard De Bélair (University Badji Mokhtar-Annaba). A voucher specimen was deposited at the herbarium of the Laboratory of Therapeutic Substances, Faculty of Sciences, Mentouri-University, Constantine, Algeria (LOST ZKAK Av 04/09).

### *Essential Oil extraction*

Hydrodistillation of fresh aerial parts of *Ammi visnaga* L. (200 g) for 3 h using a Clevenger-type apparatus, yielded 1.3% (w/w) of a yellowish oil, which was dried over anhydrous sodium sulfate then stored at +4°C until tested and analyzed.

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*Gas Chromatography-Mass spectrometry*

GC analysis was performed on a Shimadzu GC17A gas chromatograph equipped with a cross-linked DB5-MS column (40 m × 0.18 mm, film thickness 0.18 µm). The oven temperature was programmed as isothermal at 60°C for 5 min, then raised to 275°C at 5°C/min and held at this temperature for 5 min. Helium was used as the carrier gas at a rate of 1 ml/min. GC/MS was performed using a Shimadzu QP5050 mass selective detector. Operating conditions were the same as for the analytical GC. The MS operating parameters were as follows: ionization potential, 70 ev; ionization current, 2 A; ion source temperature, 200ºC; resolution, 1000. scan time, 5 s; scan mass range, 40–400 u; split ratio, 1:10.

*Identification of components*

Essential oil components were identified based on their retention indices (determined with reference to a homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns with those reported in the literature (Adams, R.P. 2007; Mc Lafferty and Stauffer 1991) and with authentic compounds.

*Antibacterial activity*

The essential oil was individually used against a range of bacteria, namely *Escherichia coli ATCC 25922*, *Escherichia coli, Staphylococcus aureus ATCC 43300, Staphylococcus aureus, Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa, Enterobacter aerogenes, Klebsiella pneumoniae,* and *Morganella morganii*. The reference strains were obtained from the Pasteur Institute (Algiers). The other strains were obtained from the laboratory of bacteriology, Benbadis Hospital, Constantine, using conventional methods (clinical isolation). Susceptibility of the bacterial strains to the essential oil was investigated using the disk diffusion method and by comparing their antibiogram inhibition zones to those reported by the National Committee for Clinical Laboratory Standards (NCCLS 1993).

## Results and discussion

The hydrodistillation yielded 1.3 % of yellowish oil. 21 components were identified representing 97.3% of the essential oil with 2,2-Dimethylbutanoic acid (30.1%), isobutyl isobutyrate (14.0 %), croweacin (12.2%), linalool (12.1%), bornyl acetate (7.3%), and thymol (6.0%), as the major components (Table 1). The composition is different from the reported oil of the seeds of the species gowing In Morocco (Lamiri et al. 2001), mainly represented by linalool (70.1%) and pentylmethylbutanoate (4.3%).

**Table 1:** Chemical composition of *Ammi visnaga* L. essential oil.

| No | Compounds[a] | RI[b] | Percentage composition |
|---|---|---|---|
| 1. | α-Thujene | 930 | 1.5 |
| 2. | 3-Methylpentenol | 935 | 2.5 |
| 3. | β-Myrcene | 992 | 0.1 |
| 4. | Isobutyl isobutyrate | 1004 | 14.0 |
| 5. | Linalool | 1029 | 12.1 |
| 6. | Methylbutyl 2-methylbutaoate | 1100 | 1.2 |
| 7. | 2,2-Dimethylbutanoic acid | 1108 | 30.1 |
| 8. | α -Isophorone | 1121 | 3.8 |
| 9. | 2-Nonyne | 1143 | 1.2 |
| 10. | Hexenyl isobutanoate | 1152 | 1.6 |
| 11. | *endo*-Fenchyl acetate | 1220 | 0.2 |
| 12. | Bornyl acetate | 1289 | 7.3 |
| 13. | Thymol | 1290 | 6.0 |
| 14. | Geranyl acetate | 1381 | 1.2 |
| 15. | Lavandulyl acetate | 1439 | 1.2 |
| 16. | Citronellyl propionate | 1446 | 0.6 |
| 17. | Croweacin | 1460 | 12.2 |
| 18. | Neryl isobutanoate | 1491 | 0.1 |
| 19. | Lavandulyl 2-methylbutanoate | 1512 | 0.1 |
| 20. | α -Damascone | 1689 | 0.1 |
| 21. | (Z,E)-farnesal | 1701 | - |
| | Identified compounds | Total | 97.3 |

[a]Compounds listed in order of their RI. [b]RI (retention index) measured relative to n-alkanes ($C_6$-$C_{24}$) on the non-polar DB5-MS column.

The essential oil exhibited the best antibacterial activity against *Escherichia coli ATCC 25922*, *Escherichia coli, Staphylococcus aureus ATCC 43300* and *Pseudomonas aeruginosa* ATCC 27853 with 29 mm, 25 mm, 25 mm, 25 mm inhibition zone diameters, respectively (Table 2a-b).

**Table 2a:** Antibacterial activity of the essential oil of *Ammi visnaga* L. (inhibition zone diameters)

| Microorganism | Inhibition zone (mm) | |
|---|---|---|
| | Ampicillin (10 µg/ml) | Essential oil (128 µg/ml) |
| *Escherichia coli ATCC 25922* | 18 | 29 |
| *Escherichia coli* | - | 25 |
| *Staphylococcus aureus ATCC 43300* | 30 | 25 |
| *Staphylococcus aureus* | - | 16 |
| *Pseudomonas aeruginosa ATCC 27853* | - | 25 |
| *Pseudomonas aeruginosa* | - | 20 |
| *Enterobacter aerogenes* | - | 20 |
| *Klebsiella pneumoniae* | 14 | 23 |
| *Morganella morganii* | - | 23 |

**Table 2b:** Antibacterial activity of the essential oil of *Ammi visnaga* L. (MIC values)

| Microorganism | MIC (µg/ml) | |
|---|---|---|
| | Ampicillin (10 µg/ml) | Essential oil (128 µg/ml) |
| *Escherichia coli ATCC 25922* | 10 | 16 |
| *Escherichia coli* | - | 16 |
| *Staphylococcus aureus ATCC 43300* | 5 | 32 |
| *Staphylococcus aureus* | - | 32 |
| *Pseudomonas aeruginosa ATCC 27853* | - | 16 |
| *Pseudomonas aeruginosa* | - | 16 |
| *Enterobacter aerogenes* | - | 16 |
| *Klebsiella pneumoniae* | 32 | 16 |
| *Morganella morganii* | - | 16 |

## Conclusion

The essential oil of *Ammi visnaga* L., collected at Didouche Mourad- Constantine (North Eastern Algerian), is mainly characterized by the presence of isobutyl isobutyrate, linalool, 2,2-dimethylbutanoic acid, thymol, bornyl acetate and croweacin as the major components. The antibacterial activity of this essential oil against several microorganisms was investigated. The best antibacterial activity was obtained against *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* strains

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