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Full Length Research Paper

Variability of two essential oils of *Ammi visnaga* (L.) Lam. a traditional Tunisian medicinal plant

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This study deals with the valorization of medicinal and aromatic plants of the Tunisian flora, in order to find new bioactive natural products. The essential oil constituents from the fruits of *Ammi visnaga*, collected from two Tunisian localities, Ichkeul and Djebba the North of Tunisia, were extracted by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). Forty-one compounds were identified. Both samples showed similar chemical composition, the major components were linalool, isoamyl 2-methyl butyrate and isopentyl isovalerate.

Key words: Apiaceae, *Ammi visnaga*, essential oil composition, Gas chromatography-mass spectrometry (GC-MS) analysis.

INTRODUCTION

The Apiaceae Lindl. (Umbelliferae) family comprises about 300 to 455 genera and 3000 to 3750 species distributed in the northern hemisphere (Rechinger, 1972; Heywood, 1999). Its members include economically important vegetables (for example, carrot, parsnip, celery) and condiments (for example, coriander, anise, caraway, cumin, parsley and dill).

In addition, species of the parsley family (Apiaceae) are well known with regards to their economic importance and diversity of essential oils (Hegnauer, 1971, 1973).

Plants of the Apiaceae family possess a range of compounds with many biological activities. Some of the main properties are ability to induce apoptosis, antibacterial, hepatoprotective and vaso-relaxant activities, cyclooxygenase inhibitory effect and antitumor action (Pae et al., 2002). Among the Apiaceae, *Ammi visnaga* (L.) Lam. is an aromatic herb known in Tunisia by the name of “guabeba” and known in Arabic as "Khella" that grows wild in the Eastern Mediterranean countries and in Arabia (IUCN, 2005).

The extract of fruits of *A. visnaga* has been widely employed as herbal medicine in the treatment of coronary diseases and bronchial asthma; it has been used as an important raw material in pharmaceutical industry (Sitting, 1988; Kleeman et al., 1999). Also *A. visnaga* is well known as a source of essential oil and is especially cultivated for it therapeutic properties (diaphoretic, carminative, antispasmodic, antiseptic, tonic,) being used in traditional medicine systems in many countries. Essential oil of *A. visnaga* is known for its proprieties against coronary diseases and bronchial asthma (Rose and Hulburd, 1992; Satrani et al., 2004).

To the best of our knowledge, there is no reference about oil content and chemical composition of Tunisian *Ammi* specie. Therefore, we report here the first study

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Table 1. Climatic characteristics (temperature, sunstroke and precipitations) of studied localities where *Ammi visnaga* fruits were harvested in July 2010.

Locality	Ichkeul (Gouvernorat: Bizert)	Djebba (Gouvernorat Beja)
GPS	37°07'43.81"N, 9'40'28.49"E	36°29'01.44"N, 9°06'16;73"E
Average temperature (°C)	26.6	28.1
Humidity (%)	62.0	53.0
Precipitations (mm)	00.0	00.0

on two essential oils of *A. visnaga* collected from Ichkeul and Djebba. It would also be noteworthy to point out that the composition of essential oils is influenced by the presence of several factors, such as local, climatic, seasonal and experimental conditions (Daferera et al., 2000).

MATERIALS AND METHODS

Plant material

A. visnaga (L.) Lam., was collected during the flowering phase (July, 2010) from two locations in Northern Tunisia differing in their climatic conditions: Ichkeul (sub-humid bioclimatic stage) and Djebba (superior semi-arid bioclimatic stage) (Table 1). Plant identification was carried out by R. El Mokni, botanist at the University of Science of Bizerte. Voucher specimens were deposited at the herbarium of this University.

Essential oil extraction

The essential oils were obtained by hydrodistillation during 3 h using a Clevenger-type apparatus (European Pharmacopoeia, 1996). The yield of each essential oil was determined on average over the three replicates. These oils were dried over anhydrous sodium sulphate and kept at 4°C until analysis.

Analysis of the essential oils

Gas chromatography-mass spectrometry (GC-MS) analyses were performed on an Agilent 6890 series gas chromatograph interfaced to an Agilent 5973 *N* mass selective detector (Agilent Technologies, Little Falls, DE, USA). A vaporization injector operating at 250°C in the split mode (1:100) was used. A fused silica capillary column, 30 m × 0.25 mm ID × 0.25 µm film thickness (TRB-5MS; 5% diphenyl 95% dimethyl polydimethylsiloxane, Teknokroma, Spain) was used. The oven temperature was programmed from 45°C for 1 min and then increased at 5°C min^{-1} to 240°C, and held isothermally for 5 min. High purity helium was used as carrier gas at 30 cm s^{-1}.

Electron ionisation mass spectra in the range 35 to 550 Da were recorded at 70 eV electron energy with an ionization current of 39.6 µA. The quadrupole, source and transfer line temperatures were maintained at 150, 230 and 280°C, respectively. A solvent delay of 5 min and a turbo molecular pump (10^{-5} torr) were used. All data was recorded using a MS ChemStation (G1701CA; Rev C.00.00; Agilent Technologies). The identity of each compound was determined by comparison of its retention index (RI) relative to C_{10}-C_{24} *n*-alkanes (Adams, 2007), as well as of its spectra with the Wiley library spectral data bank (G1035B; Rev D.02.00; Agilent Technologies). For semi-quantification purposes of the samples studied (1 µl), the normalised peak area of each compound was used without any correction factors to establish abundances. For each essential oil, the RI and the peak area percentages were calculated as mean values of three injections.

RESULTS AND DISCUSSION

The essential oil was obtained by the conventional hydrodistillation from the dried fruit of *A. visnaga*. Plant material was collected during flowering phase in two different locations in Northern Tunisia, differing in their climatic conditions: Ichkeul (sub-humid bioclimatic stage) and Djebba (superior semi-arid bioclimatic stage) (Table 1). Each distillation led us to obtain 0.175 ml of each essential oil that corresponds to 0.2% yields (V/W), based on dried weight of samples.

In this work, the chemical composition of the two essential oil samples from *A. visnaga* was analysed by GC-MS. Table 2 depicts the compounds identification and their percentages (as the mean of three analysis ± SD), as well as the RI values. These values are listed in order of their elution from TRB-5MS capillary column. From the data obtained, the essential oils showed to be complex mixtures of several components, predominating non-terpene esters and oxygenated monoterpenes.

Forty one constituents were identified and represented 98.7 and 97.9% of the total fruit oil from Ichkeul and Djebba respectively. The essential oils from *A. visnaga* were characterized by high percentages of non-terpene esters (43.3 to 49.1%) and oxygenated monoterpenes (38.5 to 39.1%).

The major components identified in both samples were linalool (23.6 and 32%), isoamyl 2-methyl butyrate (24.2 and 36%) and isopentyl isovalerate (10 and 14.8%). Non-terpene esters are also in a relative high abundance in both oils, in the oil.

Despite, *A. visnaga* can be easily found wild in the Mediterrean region very few studies exist about its essential oil (Belaiche, 1979; Günaydin and Neslihan, 2004). *A. visnaga* from Marocoo (Belaiche, 1979) provides an essential oil whose main compounds are amyl isobutyrate (16%), linalool (22.7%), methyl-2-isoamyl butyrate (27.7%) and amyl valerate (about 10%). From the previous compounds only linalool is present in Tunisian oil in a slightly high percentage, 23.6% (Ichkeul) and 32.0% (Djebba). The R isomer, (R)-(–)-linalool, has

Table 2. Chemical composition (%) of the essential oils of *Ammi visnaga* collected in Northern Tunisia: Ichkeul (sub-humid bioclimatic stage) and Djebba (superior semi-arid bioclimatic stage).

Compound	RI[a]	Ichkeul	Djebba
α-Thujene	935	0.3±0.01	0.1±0.02
α-Pinène	944	1.0±0.03	0.6±0.01
Sabinene	973	0.4±0.01	0.7±0.03
β-Pinene	976	0.9±0.03	0.1±0.01
Butyl isobutyrate	982	2.5±0.02	3.0±0.03
Myrcene	994	1.2±0.04	0.2±0.01
Pentyl-proanoate	1011	1.5±0.06	0.1±0.01
Isobutyl isovalerate	1017	0.5±0.02	0.9±0.02
α-Terpinene	1021	0.2±0.01	0.1±0.01
Limonene	1029	1.0±0.05	0.7±0.02
β-Phellandrene	1031	0.1±0.01	0.1±0.01
2-Methyl butyl-2-methyl butyrate	1047	0.1±0.01	0.6±0.03
γ-Terpinene	1058	0.1±0.01	0.5±0.02
cis β-Ocimene	1068	0.1±0.01	0.2±0.03
Amyl isovalerate	1072	0.2±0.01	1.0±0.05
trans-linalool oxide	1076	0.3±0.02	0.1±0.01
cis-linalool oxide	1083	0.6±0.02	0.1±0.01
Linalool	1099	23.6±1.1	32.0±1.4
Isoamyl 2-methyl butyrate	1107	24.2±1.3	36.0±1.07
Isopentyl isovalerate	1110	14.8±1.2	10.0±1.05
Ipsedienol	1132	0.5±0.02	0.4±0.02
α-Terpinol	1167	0.3±0.01	0.2±0.01
Lavandyl acetate	1192	0.2±0.01	0.1±0.01
β-Bourbonene	1234	1.4±0.4	0.5±0.03
β-Elemene	1238	0.1±0.01	0.1±0.01
Dodecanal	1247	1.8±0.7	0.1±0.01
Lavandyl isobutyrate	1262	2.9±0.9	0.3±0.02
Trans- bergamoptene	1277	0.2±0.01	0.2±0.01
α-Humulene	1299	0.1±0.01	0.5±0.03
Germacrene D	1325	1.6±0.05	0.8±0.02
Linalyl valerate	1389	1.5±0.07	1.0±0.06
Lavandulyl isovalerate	1402	3.4±0.2	0.4±0.01
Lavandulyl 2-methyl-butyrate	1406	2.2±0.3	0.8±0.02
β-Sesquiphellandrene	1428	0.3±0.02	1.6±0.2
Germacrene B	1458	0.1±0.01	0.1±0.01
(E) Nerolidol	1474	3.0±0.07	0.2±0.01
Spathulenol	1489	0.6±0.02	0.3±0.01
Z-Sesquilavandulol	1507	0.4±0.01	0.1±0.01
α-Bisabolol	1548	0.2±0.01	0.5±0.02
Geranyl linalool	1561	0.1±0.02	0.1±0.01
(Z) Farnesyl acetate	1583	4.2±0.05	2.5±0.03
% Identified		98.7±2.3	97.9±2.2
Non-terpene esters		43.3±2.5	49.1±2.3
Non-terpene aldehydes		1.8±0.05	0.1±0.01
Monoterpene hydrocarbons		9.8±0.7	7.3±0.5
Oxygenated monoterpenes		39.1±1.2	38.5±1.1
Sesquiterpene hydrocarbons		0.4±0.01	1.7±0.02
Oxygenated sesquiterpenes		4.3±0.04	1.2±0.01

[a] Relative to C_{10}-C_{24} n-alkanes determined using the TRB-5MS capillary column, values given are the means of three replicates ± standard deviation.

recently been claimed to repress significant changes in neutrophils and lymphocytes in rats exposed to during restraint stress for 2 h. which indicates that inhalation of this terpene attenuates stress-induced changes (Nakamura et al., 2009).

Our result also differ from the ones obtained for a essential oil from Turkish origin (Günaydin and Neslihan, 2004), which is characterized by high content of nerol (29.98%) and bisabolol (20.86%).

The variations in the percentage of chemical composition of essential oils are not very important and can be explained by the difference in the geographic origins, one a sub-humid and the other a semi-arid clima. The origin of changes should be sought mainly in the differences in the nature of soil on the one hand and solar radiation on the other. Both factors involve the activation or inactivation of certain enzymatic groups, leading to the predominance of a particular biosynthetic pathway (Satrani et al., 2004).

To our knowledge this is the first report describing the composition of Tunisian *A. visnaga* essential oil.

Conclusion

Our GC-MS studies of the essential oil from *A. visnaga* from two different localities of Northeast of Tunisia led us to identify 41 compounds. The major components were linalool, isoamyl 2-methyl butyrate, and isopentyl isovalerate. We note that the qualitative and the quantitative composition of the two studied essential oils are close, which seems to indicate that the two specimens present a similar chemotype.

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