Chemical Intra-Mediterranean Variation and Insecticidal Activity of *Crithmum maritimum*

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The chemical composition of the volatile metabolites of *Crithmum maritimum* harvested from several geographic localities along the Mediterranean coasts was studied by GC and GC-MSD. The major oil constituents were found to be dillapiole, γ-terpinene, sabinene, limonene and β-phellandrene. The Western populations were richer in dillapiole, whereas the Southern collections were characterized by increased amounts of thymol methyl ether and γ-terpinene. The Italian chemical profiles differentiated by the significant contributions of carvacrol methyl ether and isoterpinolene. The essential oils were also investigated for their insecticidal activity and their repellency against *Pheidole pallidula* (Nylander) ants and found to possess significant activity.

Introduction

Crithmum maritimum is a halophyte and chasmophyte apiaceous plant, which grows on all the world's coastlines but is particularly abundant in the Mediterranean countries (Coiffard *et al.*, 1993). It is also referred to as rock sapphire and was well known to sailors since ancient years for its anti-scurvy properties (Guerrero *et al.*, 1996).

Several reports have been published recently studying both the nutritional and toxic factors derived from this wild growing edible plant (Guil *et al.*, 1997; Guil-Guerrero and Rodriguez-Garcia, 1999; Coiffard and De Roeck-Holtzhauer, 1994) and the chemical composition of the essential oils (Pateira *et al.*, 1999; Flamini *et al.*, 1999; Senatore and De Feo, 1994; Coiffard and De Roeck-Holtzhauer, 1991). *C. maritimum* contains high amounts of vitamin C, carotenes and flavonoids as well as several other bioactive metabolites possessing cytotoxic (Cunsolo *et al.*, 1993), digestive and diuretic properties (Ruberto and Amico, 1999).

As part of our studies towards the discovery of new bioactive secondary metabolites from plant and marine sources (Roussis *et al.*, 2000a; Roussis *et al.* 2000b; Tsoukatou *et al.*, 2000; Roussis *et al.*, 2000c), several Greek plants have been analyzed. Among a large set of the initially investigated plants, *C. maritimum*, exhibited one of the highest antifeedant and insecticidal activities against the *Pheidole pallidula* ants. Other members of the family Apiaceae (Umbelliferae) have been recently shown to exhibit antifeedant and neuro- activity against the field slug *D. reticulatum* (Dodds *et al.*, 1999).

In continuation of our investigations on plant – insect interactions we decided to study apart from the chemical composition and geographic variability of *C. maritimum*, the bioactivity of the plant and determine the ant repellency and insecticidal activity of the essential oil. Our search was triggered by field observations that indicated the consistent repellency of the plant against the ants.

Experimental

Field sampling and vouchers

Plant material was collected during the period of June 1999 – August 2000. Specimens of *C. maritimum* for the geographic (West – East) variability studies were collected, during anthesis, from the coasts of San Sebastian (Spain), Napoli (Italy), and the island of Chios (Greece). Samples for the

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North – South comparisons were harvested only from areas of Greece (Athens and the Islands of Melos and Crete).

Voucher specimens have been deposited at the Herbarium of the Department of Pharmacognosy, University of Athens.

Sample analysis

Harvested plants (5–8 plants/location) were cut in small pieces and separately hydrodistilled for 2 h in a modified Clevenger apparatus with a water cooled receiver, in order to reduce hydrodistillation overheating artefacts. The essential oils were taken up in diethyl ether and dried over sodium sulfate (yields expressed in ml of essential oil per 100 g plant: *C. maritimum* from Chios = 1.25, *C. maritimum* from Italy = 2.12, *C. maritimum* from Spain = 0.88, *C. maritimum* from Ag. Marina = 1.36, *C. maritimum* from Melos = 1.05, *C. maritimum* from Crete = 1.98). A Hewlett-Packard HP6890 gas chromatograph equipped with a DB-1 (30 m × 0.32 mm) fused silica gel column was used for the quantification of the oils. Oven temperature was programmed as follows: 50 °C for 5 min and then up to 250 °C at 3 °C/min. Injector temperature: 200 °C. Carrier gas: He with a flow rate of 2 ml/min. Injector temperature: 250 °C. Mass spectra were obtained from GC/MS analysis on a Hewlett-Packard HP5973/HP6890 equipped with a 30 m × 0.25 min DB-5 capillary column. The samples were also analysed on a SGE CydexB column. The mass spectrometer was operating (full scan mode) in the EI mode at 70 eV. The identification of the chemical constituents was based on comparisons of their relative retention times and mass spectra with those obtained from authentic standards and/or the NIST/NBS and Wiley libraries spectra. Evaluation of the enantiomeric purity of the constituents showed that all major metabolites were present as a single isomer and did not reveal any obvious racemization during the process.

Optical rotation measurements

Optical rotation was measured on a Perkin-Elmer 341 Polarimeter.

The following results were obtained:

C. maritimum from Chios $[\alpha]_D$ +50.2°, *C. maritimum* from Spain $[\alpha]_D$ +111°, *C. maritimum* from Italy $[\alpha]_D$ +1.06°, *C. maritimum* from Agia Marina $[\alpha]_D$ +56.7°, *C. maritimum* from Melos $[\alpha]_D$ +36.6°, *C. maritimum* from Crete $[\alpha]_D$ +86.2°.

Ant Repellency: The repellency of the oils was tested in the field on busy (more than 20 ants per minute) ant trails. Quantities of the oils (1.0 mg, 100.0 μg, 10.0 μg) were applied, in diethyl ether solutions, on 10 mm filter paper disks. The disks were aerated for 2 min in order to allow the evaporation of the solvent. The impregnated disks along with the blanks were placed on several *P. pallidula* trails during different periods of the day. The activity was evaluated on the basis of the ability of the blends to repel the ants and forced them to diverge from their trails.

Insecticidal activity: Quantities of each essential oil (100 μl of a 250 mg/ml stock solution) were applied on filter paper disks (diam. 3 cm) so that the surface density would approximate the natural concentration (approx 1%). The disks after evaporation of the solvents were impregnated with saturated sugar solution and were placed in Petri dishes along with 21 *P. pallidula* ants. Control dishes were prepared in a similar way. The mortality was recorded daily.

Results and Discussion

A total of 61 plants were selected for this study. More than 35 metabolites were detected, characterized and quantified on the basis of their retention times and mass spectra, as constituents of the essential oils (Table I). As expected the majority of the identified metabolites were found to be monoterpenes (Jose *et al.*, 1992; Senatore and De Feo, 1994; Katsouri *et al.*, 1999). Sesquiterpenes never exceeded 3% of the total oil, which was in accordance with previously reported data (Barroso *et al.*, 1992). The main constituents of the essential oils were sabinene and γ-terpinene that were always present, limonene and β-phellandrene that were absent in the Italian sample, dillapiole that was never detected in the Greek samples and thymol methyl ether that reached the higher amounts in the samples collected from Crete and San Sebastian. Carvacrol methyl ether, on the other hand, was detected in considerable amounts in the samples from Chios and Napoli that was further differentiated by the presence of isoterpinolene. Guido *et al.* (1999) suggested that sabinene is pro-

Table I. Percentage contribution of the identified metabolites of *Crithmum maritimum* essential oils.

		East	⟶	**West**	**North**	⟶	**South**
Compound	**Kovats Index**	**Chios**	**Italy**	**Spain**	**Agia Marina**	**Melos**	**Crete**
Tricyclene	926		0.39 (0.12)				
α-Thujene	931	0.39 (0.041)*		0.20 (0.0011)	0.33 (0.095)	0.16 (0.11)	0.22 (0.058)
α-Pinene	939	1.91 (0.22)	0.38 (0.012)	1.21 (0.32)	0.74 (0.074)	7.69 (0.42)	0.68 (0.041)
α-Fenchene	951	traces					
Camphene	953					traces	
Sabinene	976	15.1 (2.4)	0.98 (1.1)	8.07 (0.15)	38.0 (4.1)	3.28 (0.85)	21.8 (0.76)
β-Pinene	980	2.81 (0.16)					
Myrcene	991	1.60 (0.26)	0.55 (0.065)	0.43 (1.4)	1.62 (0.38)	0.85 (0.43)	0.56 (0.074)
α-Phellandrene	1005	0.55 (0.023)			0.51 (0.19)	0.21 (1.2)	
α-Terpinene	1018	0.58 (0.078)	0.45 (0.32)	0.36 (0.034)	1.00 (0.05)		0.42 (0.12)
o-Cymene	1022		6.23 (0.41)	2.21 (0.98)			
p-Cymene	1026	1.65 (0.05)	0.94 (0.12)		1.80 (0.54)		6.43 (0.78)
Limonene	1031	20.4 (2.0)			12.8 (0.46)	28.5 (1.4)	
β-Phellandrene	1031	8.62 (2.4)		0.15 (0.45)	18.5 (2.6)		11.3 (1.4)
cis-Ocimene	1040	8.11 (0.73)		0.35 (0.026)	0.72 (0.15)	6.53 (0.66)	
trans-Ocimene	1050	0.66 (0.078)				0.24 (0.061)	0.85 (0.058)
γ-Terpinene	1062	22.8 (4.8)	39.4 (1.7)	14.9 (2.6)	17.1 (2.5)	33.3 (4.6)	35.4 (2.9)
trans-4-Thujanol	1068	0.19 (0.15)		0.13 (0.21)	0.42 (0.11)		
Isoterpinolene	1086	0.22 (0.095)	6.54 (0.85)		0.36 (0.12)		
Terpinolene	1088			0.21 (0.078)		traces	0.13 (0.015)
cis-4-Thujanol	1097	0.13 (0.05)	0.15 (0.11)		0.42 (0.068)		traces
cis-*p*-Menth-2-en-1-ol	1121				0.57 (0.072)		
Allo-ocimene	1129	0.25 (0.14)				traces	
Terpin-4-ol	1177	2.55 (0.45)		1.91 (0.26)	3.87 (0.16)	0.26	1.76
α-Terpineol	1189			0.30 (0.043)			
Elemicin	1189			0.41 (0.16)			
Thymol methyl ether	1235	0.25 (0.095)	0.61 (0.089)	20.4 (1.8)	0.32 (0.25)	13.7 (2.9)	19.7 (1.7)
Carvacrol methyl ether	1244	6.23 (0.75)	25.8 (6.4)	0.12 (0.11)	0.66 (0.045)		
Bornyl acetate	1285			0.20 (0.089)			
trans-caryophyllene	1418	traces				0.13 (0.25)	
γ-Muurolene	1477	0.99 (1.0)					
Bicyclogermacrene	1494	0.81 (0.096)		0.17 (0.061)		2.78 (0.56)	
Myristicin	1520			1.20 (0.56)			
δ-Cadinene	1524	traces					
Spathulenol	1576	0.11 (0.36)				0.17 (0.25)	
Dillapiole	1622		11.50 (1.15)	35.1 (4.3)			
Farnesol	1697					0.24 (0.056)	
% Total identified metabolites		97	94	88	99	98	99

* The numbers in parentheses represent the standard deviations.

duced in higher amounts only during fruit ripening. Dillapiole, the only phenylpropane derivative identified in the oils, is a frequently detected component of *C. maritimum* (Pateira *et al.*, 1999; Coiffard *et al.*, 1993). These later compounds along with thymol methyl ether were shown to possess significant activity against (+)-Gram and (−)-Gram bacteria and certain fungi (Ghani and Hafez, 1995; Guido *et al.*, 1999).

Regarding the geographic variation of *C. maritimum* chemical composition an interesting pattern was observed and several observations were made. Noteworthy is the prominent increase in the oxygenated terpenes from East to West as well as from North to South. Samples collected from the East Mediterranean coasts were richer in terpenes, namely limonene and β-phellandrene. These two monoterpenes exhibited a remarkable variation from North to South. They were both present at 23% and 12%, respectively, in the samples collected from the Northern sites (Agia Marina) while at the southern sites only limonene (Melos, 28%) or β-phellandrene (Crete, 11%) were detected. Sabinene content decreased from North to South, while both γ-terpinene and thymol methyl ether content increased. Finally, o-cymene and dillapiole were present only in the West Mediterranean plants.

In the course of the present study, the ant-repellant activity of the various *C. maritimum* oils was assayed. The field experiments showed that all essential oils were very active. The 1.0 mg disks forced the ants to abandon completely the trails whereas the lower concentrations kept their repellency for at least 4 hours. Since the ants perceive the most volatile metabolites, it is believed that the headspace components must be those responsible for the repellent properties of the plants. Furthermore, the insecticidal activity was studied. The essential oils showed very high toxicity and in all assays 90% mortality was measured within 3 days (Table II). Especially the Spanish and Italian essential oils exerted higher mortality rates. Since dillapiole was one of the main contributors in these oils it is presumed that it might be one of the active constituents of the oils.

Table II. Insecticidal activity*,# of *C. maritimum* essential oils.

Application	Insect mortality (Dead ants)						
Days	**Control**	**Spain**	**Italy**	**Ag. Marina**	**Melos**	**Crete**	**Chios**
1	0	8	8	5	6	7	7
2	1	17	14	11	14	12	12
3	1	20	17	15	18	16	12
4	2	20	19	17	18	20	16
5	4	21	21	17	19	20	18
6	4			18	21	21	20

*: Number of assayed *P.pallidula* per essential oil: 21.
#: The ant assays were repeated seven times.

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