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ESSENTIAL OILS FROM WILD AND MICROPROPAGATED PLANTS OF *ORIGANUM BASTETANUM*

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Key Word Index—*Origanum bastetanum*; Lamiaceae; essential oils; *cis*-4-thujanol; γ-terpinene; thymol; *p*-cymene; micropropagation.

Abstract—We analyzed water-distilled essential oil of *Origanum bastetanum*, an endemic species in Spain, by gas chromatography/mass spectrometry. Twenty-five components were characterized; *cis*-4-thujanol (30.9%), γ-terpinene (15.6%), thymol (12.0%), *p*-cymene (9.9%), 1-terpinen-4-ol (5.9%) and linalyl acetate (3.7%) were identified as major constituents. We also studied the essential oil of micropropagated *O. bastetanum* and the results were compared with those from wild plants. Micropropagated plants can be used to repopulate original localizations and other areas of south east Spain.

INTRODUCTION

Oregano (*Origanum* L. sp. pl.), is widely used as a medicine and food. The essential oils of several oregano species have expectorant, antispasmodic, tonic, antiseptic, analgesic, antimicrobial, antifungic, antioxidant, germicidal and cytotoxic properties [1–6]. *Origanum bastetanum* [7], an endemic species in the south eastern Iberian Peninsula (NE of the province of Granada, Eastern Andalusia) exhibits these properties and is also of interest because its distribution partly coincides with the area of the European Union LUCDEME program (Fight Against Desertization of the Mediterranean Area). This species is adapted to erosive conditions due to its well-developed root system. Current techniques for obtaining a large number of plants by micropropagation of seeds are extremely effective and this allows eroded areas to be resown.

In a previous study, we used micropropagation of seeds of *Sideritis foetens* [8] to obtain plants whose chemical composition was similar to that of wild plant, and which were successfully sown in different areas of Alcolea (Almería). Here we describe micropropagation from seeds of *O. bastetanum*, another endemic species of south east Spain. We analysed the essential oils of the aerial parts of wild and micropropagated plants, and compared the results with those published for other oregano species.

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RESULTS AND DISCUSSION

Essential oils were obtained by distillation in water of the aerial parts of *O. bastetanum* collected from a wild population [7] and from the plants obtained by micropropagation of seeds from the same population. Essential oils were analyzed as described in the Experimental. Table 1 summarizes the results of the analyses of the essential oils and shows the relative composition with regard to total oil content. In addition, the essential oils of wild *O. bastetanum* were separated chromatographically and some of the major components, *cis*-4-thujanol, 1-terpinen-4-ol and thymol, were identified by comparison with the spectroscopic data reported in literature [9, 10].

Micropropagated plants produced smaller yields of essential oil and its composition was slightly different from that of wild *O. bastetanum*. The major components of the essential oil from micropropagated plants were γ-terpinene (31.9%), *p*-cymene (17.6%), thymol (16.5%) and *trans*-caryophyllene (9.4%). However, the major components of the essential oil from wild *O. bastetanum* were *cis*-4-thujanol (30.9%), γ-terpinene (15.6%), thymol (12.0%), *p*-cymene (9.9%), 1-terpinen-4-ol (5.9%) and linalyl acetate (3.7%). In both cases, the carvacrol content was less than 0.7%.

Analyses of the essential oils from different species of oregano have shown that thymol and carvacrol are the main components [11]. In *O. vulgare* ssp. *hirtum* and *O. dictamus*, thymol, carvacrol, γ-terpinene and

Table 1. Composition of essential oils of wild and micropropagated *Origanum bastetanum*

Compound	% in Oil	
	Wild	Micropropagated
α-Pinene	tr	2.1
Camphene	—	0.9
Sabinene + β-Pinene	2.5	tr
Myrcene	2.2	1.6
α-Phellandrene	—	1.0
p-Cymene	9.9	17.6
α-Terpinene	—	2.1
γ-Terpinene	15.6	31.9
trans-Thujanol	1.0	—
Terpinolene	1.1	tr
cis-Thujanol	30.9	—
Linalool	1.0	tr
Terpinen-1-ol	0.5	—
endo-Borneol	tr	0.4
1-Terpinen-4-ol	5.9	2.4
α-Terpineol	2.7	0.5
Thymyl methyl ether	0.3	1.0
Carvacryl methyl ether	0.4	2.2
Linalyl acetate	3.7	tr
Thymol	12.0	16.5
Carvacrol	0.2	0.6
Thymyl acetate	—	0.2
Neryl acetate	0.3	tr
Geranyl acetate	0.1	tr
β-Bourbonene	—	0.6
trans-Caryophyllene	2.1	9.4
α-Humulene	—	1.5
Germacrene D	—	0.7
α-Farnesene	0.3	0.8
β-Bisabolene	—	2.0
γ-Cadinene	0.1	0.2
δ-Cadinene	0.2	0.4
Germacrene B	2.4	tr
Spathulenol	1.3	0.9
Caryophyllene oxide	0.2	1.1

p-cymene constituted 73.7% and 92.8%, respectively, of the total oil. The proportion of thymol and carvacrol varies in different oregano species: 30:30 in *O. syriacum* [4]; 20:37 in *O. glaucum* [12]; and 19:41 in *O. heracleoticum* [13]. In some species, the carvacrol content is considerably higher, e.g. 72% in *O. smyrnaeum* [14] and 65.88% in *O. bilgeri* [15]. Nevertheless, in other species of oregano, thymol and carvacrol are not the major components. The essential oil of the leaves of *O. dubium* [16] consisted of 1,8-cineol (32.2%), linalool (35.5%) and camphor (7.02%). In *O. laevigatum* [17], the major components were bicyclogermacrene (37.9%), germacrene D (21.7%) and β-caryophyllene (4.5%). In *O. vulgare* from northern India, linalool (23.8%), myrcene (18%), β-caryophyllene (9.06%), germacrene D (7.4%) and 1-terpinen-4-ol (4.4%) were the major constituents [18], whereas in *O. micranthum* [19] the major components were linalyl acetate (12.3%), *cis*-4-thujanol (10.8%), α-terpineol (9.67%), linalool (8.8%) and 1-terpinen-4-ol (8.73%).

Our results show that in wild *O. bastetanum*, the chemical composition of its essential oil is similar to that of *O. micranthum*, an endemic species from Turkey, although the content of *cis*-4-thujanol is considerably higher in the Spanish species. Its composition is different from that of the oils from other geographically and taxonomically related oreganos, such as *O. vulgare* and *O. virens* [11]. The difference in composition between the wild *O. bastetanum* and plants micropropagated from their seeds may be due to the different environmental conditions the two were subjected to. Because it is an endemic species in the process of extinction, we have investigated the acclimatization of plants obtained by micropropagation in the original location and in other locations with different ecological conditions.

EXPERIMENTAL

Material

Wild *O. bastetanum* Socorro, Arrebola and Espinar was collected during the flowering period in July 1996 in Benamaurel (Granada) (30SWG26, 700 m, O. Socorro and A. Martínez, GDA 22536). Micropropagated plants of *O. bastetanum* were collected during the flowering period from the shade-house of the Centro de Investigación y Desarrollo Agrario (CIDA) in Churriana (Málaga).

Germination, multiplication, growth and acclimatisation

Seeds of wild *O. bastetanum* collected in the ripening period were used as starting material. Seeds were disinfected with an aq. soln of 0.5% NaOCl for 5 min and washed × 3 with sterile H_2O for 5 min each. They were then treated with an aq. soln of GA_3 (0.57 mM) for variable periods, although the best germination rate (95%) was obtained without GA_3 treatment. Seeds were incubated for 5 weeks. The culture medium [20] consisted of 87.64 mM sucrose, 55.49 10^{-3} mM *m*-inositol, 2.96 10^{-3} mM thiamine HCl, 2.43 10^{-3} mM pyridoxine HCl, 4.06 10^{-3} mM nicotinic acid and 26.63 10^{-2} mM glycine, sterilized at 121° for 15 min (pH 5.74). The culture medium (25 ml) was poured into 25 × 150 mm tubes, which were placed in Heller's racks [21]. Then one seed was placed in each tube, capped with polypropylene stoppers and incubated in a culture chamber at 24 ± 1°, under a photon density of 40 ± 4 μE m^{-2} s^{-1} (Sylvania Gro-lux, fluorescent lamps) with 16 h photoperiods.

Stems of small plants obtained after germination were separated into nodal sections with two axillary buds and implanted in a multiplication medium of the same composition as described above, although solidified by the addition of Bacto-agar (0.8%) and

0.22 μM BA. This concentration of BA was selected after several experiments to evaluate the best relation between length of the main stem, number and length of axillary stems, number of leaves and development of the root system. Stems were incubated for 4 weeks in the same conditions as seeds until a total of three subcultivations. Rooting was done *in vivo* with microstakes (1.5 cm) on peat Substrate-1 (Triohum) under nebulization at room temp. (15–35°) and relative humidity 90–100% for 3 weeks, and then in a polyethylene tunnel without nebulization for 2 weeks. After this time, plants were sown in 9 cm diameter pots and maintained at 55–60% humidity for 4 weeks. Finally they were transferred to 16 cm diameter pots with blond, peat, soil-sand (2:2:1) in a shade-house. The plants remained in the shade-house until the flowering period, when the study material was collected.

Essential oil

Plant material of wild *O. bastetanum* (20 g) was distilled in a Clevenger-type apparatus for 5 h and 0.5 ml of essential oil was obtained. Under the same conditions, plant material of micropropagated *O. bastetanum* (20 g) yielded 0.3 ml of essential oil. The oils were collected in 1 ml of pentane, dried ($MgSO_4$) and stored at 4–6°. Essential oils were analyzed by GC/MS.

Analyses

Essential oils were analyzed on a 30 m × 0.2 mm i.d. cross-linked 5% methyl-phenyl siloxane column. Injector temp. was 230° in splitless mode. The column temp. was programmed at 50–320° at 9 min^{-1}. The carrier gas was He at a flow rate of 1 ml min^{-1}. Mass spectra were obtained at 70 eV and individual GC peaks were identified by a computer search of the Wiley Library 6th edition, followed by matching of the MS data and comparison, in some cases, of their R_ts with those of standards. The essential oil of wild *O. bastetanum* was chromatographed on a silica gel column eluted with pentane and pentane-Et_2O mixts. Four frs were obtained and analyzed by GC/MS. ^1H NMR spectra of the second, third and fourth frs were obtained and thymol (95%), 1-terpinen-4-ol (62%) and *cis*-4-thujanol (84%) were identified as the major components of these frs by comparison of ^1H NMR data with previously reported values [9, 10].

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