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Seasonal Variation in the Volatile Oil of *Myoporum laetum* Leaves

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ABSTRACT

Myoporum laetum (Myoporaceae), an evergreen shrub, is cultivated in Egypt as an ornamental plant and flowers from May to June. In order to study the changes of the essential oil (EO) percentage and its main components due to seasonal variation, four samples were taken from the same tree in February, May, August and November. EO percent was determined separately in each sample by hydro-distillation. The EO percentage was 0.12, 0.18, 0.23 and 0.13%, respectively. GC/MS analysis of the resulting EO revealed the presence of 27 compounds, the major one being elemicin, a sesquiterpene, which ranged from 16.6 to 50%. Ngaione, the second major compound, a furanoid sesquiterpene ketone, ranged from 26 to 44.7% (v/w) of the EO. There was a clear effect by season on EO production and its chemical composition. The major constituents of the EOs were sesquiterpenes. The oxygenated compounds were more than non-oxygenated ones in all samples.

Keywords: essential oil, sesquiterpenes, variation in volatile leaf oil

INTRODUCTION

Myoporaceae is a relatively small family and consists of three genera, one of which is *Myoporum*. *Myoporum laetum* is an evergreen ornamental shrub that flowers from May to June. A group of furanoid sesquiterpene ketones, which are essentially oxygenated farnesols, characterize the essential oil (EO) of a number of *Myoporum* species (Hegatty *et al*. 1970; Blackburne *et al*. 1972a, 1972b; Sutherland and Rodwell 1989). The best known of these is (-)-ngaione, the first isolated from a New Zealand species of *M. laetum* in 1925 (McDowell 1925), from *M. ucuminatum* (Birch *et al.* 1953) and from *M. deserti* (Hegatty *et al*. 1970). Other similar sesquiterpenes have been isolated from races of *M. deserti* and from *M. ucuminatum*. These include myodesmone and isomyodesmone with their presumed precursor, (-)myoporone, and also 10,11-dehydromyoporone (Blackburne *et al.* 1971). A sesquiterpene alcohol was characterized from the wood EO of *M. crassifolium* (O'Donnell and Sutherland 1989). Iridoid was isolated from *M. deserti* (Grant *et al*. 1980, 1985). Later, EO of the leaves of *M. laetum* was fractionated, identified and showed antiviral and antibacterial activity (Ibrahim *et al*. 2006). Several flavones were identified in *M. tenuifolium* (Tomas *et al*. 1985).

This work aimed to study seasonal variations in the EO content of *M. laetum* leaves and its main constituents.

MATERIALS AND METHODS

Plant materials

M. laetum leaves were collected from trees growing in the Faculty of Agriculture, Cairo University, Giza, Egypt. The tree was identified by Mrs. Tersea Labib, a taxonomist at the Ormamental Botanical Garden, Giza. A voucher specimen was deposited at the Herbarium of the National Research Center, Dookki, Giza, Egypt (No. 13245). Four samples from the leaves of the same tree were collected on the 15th November 2005, 15th February 2006, 15th May 2006 and 15th August 2006. The fresh samples were immediately and separately subjected to EO extraction.

Essential oil extraction

The EO of each sample was separately extracted from the fresh leaves of each sample by hydrodistillation using a Clevenger-type apparatus as described by Günter (1961), in which 2 ml of *n*-hexane was added to the tube of the apparatus as extracting solvent because the EO was heavier than water. The extracted EOs were dried over anhydrous sodium sulphate and kept in a refrigerator until Gas Chromatograph-Mass Spectrometer (GC-MS) analyses. The EO content of each sample was determined in triplicate and the mean values were calculated. The resulting EOs were subjected to GC/MS using a Hewlett-Packard GC/MS spectrometer, model 5970 under the following conditions: fused silica capillary column (50 m × 0.32 mm) coated with Carbowax 20M. Oven temperature was programmed: 60-200°C increasing by 3°C/min; He carrier gas with flow rate of 1 ml/min; injection temperature 150°C, TIC detector. MS ionization voltage 70 eV. Qualitative and quantitative identification of the EO constituents was carried out by comparing the mass fragmentation patterns with computer matches and previously published data (McLafferty and Stauffer 1991; Adams 1995; Ibrahim *et al*. 2006).

RESULTS AND DISCUSSION

Metabolic processes affect the accumulation of secondary metabolites, i.e. volatile oil or EO, in plants and is controlled by the physiological age of the plant and by environmental conditions. Thus, it is important to study the EO content and its main constituents in the leaves of a plant through the year to determine the best time to collect leaves for EO extraction. The mean values of EO percentage of the fresh leaves collected on the four collection dates were 0.12, 0.18 and 0.23 and 0.13%, respectively. The EO% fluctuated and reached a peak in May, the peak of the growth season (**Table 1**). This may be attributed to more metabolites in leaves as a result of the activity of plants in spring and summer. Omer *et al*. (1993) also found that the EO of *Schinus terebenthiflius* leaves showed a maximum value in April and a minimum in January, corresponding to spring and winter months, respectively. Husain *et al*. (1988) stated that the EO% of eucalyptus (*Eucalyptus globulus*) fresh leaves

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Short Communication

Table 1 The relative percentages of the main constituents of the essential oil of *Myoporum laetum* leaves at different seasons as separated by GC/MS.

RT	Component	M^+	B.P.	15 November	15 February	15 May	15 August
5.72	Ethanol	46	31	0.89	0.00	0.00	0.11
6.19	2-Pentanone-4-methyl	100	43	0.15	0.13	0.16	0.00
12.35	1-Hexanol	102	56	0.29	0.23	0.06	0.00
13.26	3-Hexene-1-ol	100	41	0.37	0.00	0.00	0.22
15.07	1-Octen-3-ol	128	57	0.00	0.75	6.33	4.30
15.79	Benzaldehyde	106	105	0.32	0.41	0.00	0.17
26.06	Cubebene	204	161	1.20	0.43	4.75	3.98
27.98	B-Elemene	204	67	12.24	6.94	0.55	1.48
29.12	*Trans*-Caryophyllene	204	41	5.95	3.50	0.87	1.25
29.57	Epi-bicyclosesquiphellandrene	204	161	0.15	0.00	0.00	0.00
30.14	Farnesene	204	41	0.11	0.00	0.00	0.00
30.61	Humulene	204	43	1.80	1.52	0.79	0.45
31.51	Unknown	--	--	0.09	0.20	0.26	0.07
31.75	Germacrene-D	204	161	5.98	2.25	1.07	0.71
32.03	α-Cedrene	204	119	0.56	1.56	1.08	0.12
32.25	Unknown	--	--	0.32	0.25	0.99	0.23
32.64	*Trans*-Bisobolene	204	93	3.25	0.00	0.00	0.71
33.26	δ-Cadinene	204	119	1.31	1.56	1.23	0.12
34.84	Elemicin	208	208	16.64	31.01	27.77	50.18
38.8	α-Cadinol	222	43	0.13	0.17	0.75	0.45
38.69	Muurolol	222	95	0.49	0.36	1.12	0.08
39.49	Myomontanone	232	95	2.41	1.47	0.40	0.72
40.56	Ngaione	250	151	35.75	44.71	32.52	26.04
40.74	Unknown	--	--	0.91	0.20	0.51	2.12
41.74	Unknown	--	--	0.22	0.79	1.13	0.36
44.93	Dehydromyoporone	288	83	7.1	0.41	13.46	6.23
46.72	Myoporone	250	95	1.37	1.15	4.20	0.73
	Oxygenated compounds			65.91	80.80	86.77	89.23
	Non-oxygenated compounds			32.55	17.76	10.34	7.99
	Total identified compounds			98.46	98.56	97.11	97.22
	Non identified compounds			1.54	1.44	2.89	2.78

dropped to a minimum in winter. Ibrahim and Amer (1992) found that the maximum EO% of *Callistemon lanceolates* leaves was observed during flower development, while the minimum was observed during the rest period of the tree (January and February).

The qualitative and quantitative results of the main constituents of the EOs of the four seasonal samples are shown in **Table 1**. GC-MS analyses of the EOs reveal that the major compound was elemicin (a sesquiterpene) and ranged from 50.18% (in August) to 16.64% (in November). Ngaione, also a sesquiterpene, was the second major compound with a relative percentage that ranged from 26% (in August) to 44.7% (in February). The oxygenated compounds ranged from 89.2% in August to 65.9% in November, while the non-oxygenated compounds showed an inverse pattern, ranging from 32.5% in November to 7.99% in August. This implies that the terpenoid oxygenated hydrocarbons are the major constituents in all samples. The changes in the different constituents due to season may be attributed to the effect of climatic (non-edaphic) conditions i.e. temperature, relative humidity and light on metabolic processes and on enzymatic systems of terpenoid biosynthesis (Firmage 1981; Ibrahim 1989). As shown in **Table 1** most compounds are sesquiterpenes that ranged from 97.0% in February to 90.6% in May.

We conclude that the best time for collection of EO from *Myoporum laetum* leaves is May.

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