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Essential oil composition of *Pistacia lentiscus* L. and *Myrtus communis* L.: Evaluation of antioxidant capacity of methanolic extracts

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Abstract

The seasonal variation of the essential oil composition, the antioxidant activity (DPPH, FRAP assays) and the total phenolic content (Folin-Ciocalteu assay) of two aromatic wild plants, *Pistacia lentiscus* L. (Anacardiaceae) and *Myrtus communis* L. (Myrtaceae), grown in Zakynthos, a Greek island, was investigated. The essential oil was obtained by hydrodistillation and subsequently analysed by GC–MS.

The essential oil composition of *P. lentiscus* L. was characterised by a high monoterpene hydrocarbon fraction (45.0–68.3%), which was found in greater amounts during the flowering stage (May). At the same stage, the extracts showed the highest free radical-scavenging activity ($IC_{50} = 5.09$ mg/l) and antioxidant capacity (131 mmol/l), as well as the highest phenolic content (588 mg gallic acid/g plant material). The strongest antioxidant activity and the highest phenolic content for *M. communis* L. were obtained during full flowering stage (August). Its essential oil composition was characterised by a high oxygenated monoterpene fraction (70.1–73.2%), the highest accumulation of which was also observed during the same flowering stage.

Keywords: Seasonal variation; Essential oils analysis; Antioxidant activity; Phenolics; *Pistacia lentiscus* L.; *Myrtus communis* L.

1. Introduction

Pistacia lentiscus L., which belongs to the family Anacardiaceae, is a dense bush with a strong characteristic aroma and green leaves, which grows in many Mediterranean countries (Zrira, Elamrani, & Benjilali, 2003). The aerial part of *P. lentiscus* L. has traditionally been used in the treatment of hypertension and possesses stimulant and diuretic properties (Bentley & Trimen, 1980). Some researchers reported the chemical composition of the essential oil from leaves of *P. lentiscus* L. of diverse origins (Douissa et al., 2005). However, there is only a small number of reports available in the literature, studying the antioxidant properties of the methanolic plant extract by DPPH (Baratto et al., 2003) and FRAP assay (Ljubuncic, Azaizeh, Portnaya, Coganc, & Said, 2005), as well as the total phenolic content estimated by the Folin-Ciocalteu assay (Stocker et al., 2004).

Myrtus communis L. (Myrtaceae) is an evergreen scrub, typical of the Mediterranean maquis, which grows spontaneously in many countries. It is traditionally used as an antiseptic, disinfectant drug and hypoglycaemic agent (Elfellah, Akhter, & Khan, 1984). Different parts of the plant find various uses in the food industry, such as for flavouring meat and sauces, and in the cosmetic industry (Chalchat, Garry, & Michet, 1998). The chemical composition of the

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essential oil of the plant has been reported by other researchers (Boelens & Jimenez, 1991, 1992; Bradesi, Tomi, Casanova, Costa, & Bernardini, 1997; Chalchat et al., 1998; Koukos, Papadopoulou, Papagiannopoulos, & Patiaka, 2001; Ozek, Demirci, & Baser, 2000). A classification of *M. communis* L. essential oil was proposed by Bradesi et al. (1997). According to them, myrtle oil can be categorised in two groups (chemotypes), on the basis of myrtenyl acetate content. Each group can be further divided into two subgroups, according to the relative ratio of α-pinene to myrtenyl acetate or α-pinene to cineole. Investigation of the free radical-scavenging activity of the methanolic plant extract was carried out using the TEAC assay (Montoro et al., 2006). The antioxidant activity of myrtle liquor was reported by Alamanni and Cossu (2004).

The presence of phenolic compounds (phenolic acids, polyphenols and flavonoids) in herbs and spices, along with the essential oils, is gaining increasing attention because of their various functions, such as antioxidant activity and flavouring properties (Issa, Volate, & Wargovich, 2006; Lagouri, Blekas, Tsimidou, Kokkini, & Boskou, 1993; Lagouri & Boskou, 1995; Sacchetti et al., 2005; Tsimidou & Boskou, 1994). Consumption of food containing natural essential oils or aromatic plant extracts is expected to prevent the risk of many free radical-mediated diseases (Milan, 2006; Young & Woodside, 2001). The plants under study have not generally received much attention as antioxidants and flavouring sources; because of their low yield they have little commercial use (Bradesi et al., 1997; Soong & Barlow, 2004; Zrira et al., 2003).

This paper deals with the seasonal variation of the essential oil composition of *P. lentiscus* L. and *M. communis* L., grown in Zakynthos (West Greece). Furthermore, the antioxidant activity with DPPH and FRAP and the total phenolic content with Folin-Ciocalteu of the methanolic plant extracts were studied.

The aim of our research, which is carried out for the first time for these two plants grown in Greece, is to acquire valuable information about the best harvesting period (highest concentration of the major oil components) and the relationship between the concentration of the phenolic components and the antioxidant activity of extracts.

2. Materials and methods

2.1. Plant material

The aerial parts of *M. communis* L. (Myrtaceae) and *P. lentiscus* L. (Anacardiaceae) were collected at the end of (a) February, (b) May and (c) August of 2005, in Zakynthos (a Greek island located on the Ionian Sea). Only the leaves were analysed. Full details are provided in Table 1. The freshly-cut plants were dried in a dry and shady place at ambient temperature for one month, packed in paper bags under N_2 and stored at ambient temperature. All samples were analysed within 3 months of collection.

2.2. Reagents and standards

All solvents and reagents were of the highest purity needed for each application. Folin-Ciocalteu reagent, methanol p.a, hydrochloric acid (37%), ferric chloride 6-hydrate and sodium acetate trihydrate were purchased from Merck (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH, 98%) and gallic acid (99%) were purchased from Sigma–Aldrich (Steinheim, Germany). Sodium carbonate anhydrous and 2,4,6-tris(2-pyridyl)-*S*-triazine (TPTZ, ⩾99%) were obtained from Fluka (Buchs, Germany). Glacial acetic acid was purchased from SDS (Penien, France) and dichloromethane (p.a) was purchased from Lab-Scan (Dublin, Ireland). The chemical constituents of the oils were identified by comparison with reference compounds purchased from Fluka, Acros Organics (Geel, Belgium) and Sigma–Aldrich.

2.3. Preparation of the extracts

Methanolic extracts were obtained as follows: 30 ml of aqueous methanol (70:30 v/v) containing BHT (1 g/l) were added to 0.5 g of plant material in a 50 ml round-bottomed flask. Then, 10 ml of 6 M HCl were added carefully and the mixture was stirred and sonicated for 15 min. After sonication, the mixture was bubbled for 40–60 s with N_2 and refluxed in a water bath at 90 °C for 2 h. After cooling in the dark, it was filtered and made up to 100 ml with meth-

Table 1
Collection data[a] for the plants used in the study

Plant	Location in Zakynthos island	Vegetative stage	Month	Temperature (°C)	Humidity (%)	Rainfall (mm)	Altitude (m)
Pistacia lentiscus L. (Anacardiaceae)	South (S) of Kiliomenos village. Pinus forest and clearings. 37°43′ 31N; 20°47′ 47E	Before flowering	February	7.9	63.1	86.6	360
		Flowering	May	17.8	66.7	11.4	
		Fruiting	August	24.1	63.1	0.0	
Myrtus communis L. (Myrtaceae)	East (E) of Lithakia village. Olive groves. 37°42′ 59N; 20°50′ 36E	Fruiting	February	7.9	63.1	86.6	20
		Before flowering	May	17.8	66.7	11.4	
		Full flowering	August	24.1	63.1	0.0	

[a] Year 2005.

anol. The final solution was used for the determination of antioxidant activity and total phenolic content.

Essential oils were obtained as follows: 30 g of chopped leaves of each species were subjected to hydrodistillation in a Clevenger apparatus for 3 h with 300 ml of deionised water. The resulting essential oils were dried over anhydrous sodium sulfate and after filtration, they were stored at 4 °C until further analysis. The respective colours varied from light yellow for *M. communis* L. to yellow for *P. lentiscus* L. Yields (%) of the essential oils are presented in Tables 2 and 3.

2.4. Analysis of the essential oils

GC–MS analysis of the essential oils was performed using a Fisons 8000 series gas chromatograph (Model 8060) coupled to a Fisons MD 800 quadrupole mass spectrometer (Fisons Instruments, Manchester, UK). Helium was used as carrier gas at a flow rate of 1.0 ml/min. Separation of compounds was performed on a CP-Sil 8 (30 m × 0.32 mm, film thickness = 0.25 μm, Chrompack) and on a DB-Wax capillary column (30 m × 0.25 mm, film thickness = 0.25μm, J&W). Diluted samples (1/100 in dichloromethane, v/v) of 1 μl were injected manually in split mode (split ratio 1/30). Oven temperature was programmed from 40 to 250 °C at a rate of 4 °C/min and held at 250 °C for 5 min. The injector, ion source and interface temperatures were set at 230, 200 and 270 °C, respectively. The mass spectrometer was operated in electron impact mode with the electron energy set at 70 eV and a scan range of 30–400 *m*/*z*. Oil constituents were identified by comparing: (i) linear retention indices based on a homologous series of even numbered *n*-alkanes (C_8–C_{24}) (Niles, Illinois, USA) with those of standard compounds and by comparison with literature data (Adams, 2001), and (ii) MS data with those of reference compounds (Sigma–Aldrich and Acros Organics) and by MS data obtained from Wiley (http://eu.wiley.com/WileyCDA/WileyTitle/productCd-04-70047852.html) and NIST (http://www.nist.gov/srd/nist1a.htm) libraries.

2.5. Antioxidant activity

2.5.1. DPPH assay

Free radical-scavenging activity of the sample extracts was evaluated with the modified DPPH (1,1-diphenyl-2-picrylhydrazil radical) assay (Brand, Cuvelier, & Berset, 1995), which is based on the measurement of the reducing ability of antioxidants toward the DPPH radical. Briefly, a stock solution of DPPH (10^{-4} M) was prepared in aqueous methanol (70:30 v/v). One millilitre of sample was added to 3 ml of the DPPH solution. The mixture was then shaken vigorously and allowed to stand at room temperature in the dark. After 30 min, the decrease in absorbance at 517 nm was measured against a blank (aqueous methanol solution) by using a double-beam UV–vis spectrophotometer (Jasco V-530, Tokyo, Japan). A mixture consisting of 1 ml of aqueous methanol (70:30 v/v) and 3 ml of DPPH solution was used as the control. The radical stock solutions were freshly prepared every day, stored in a flask covered with aluminium foil and kept in the dark.

The radical-scavenging activities of the samples, expressed as percentage inhibition of DPPH, were calculated according to the formula

$$\%\ \text{Inhibition} = [(A_B - A_A)/A_B] * 100$$

where A_B and A_A are the absorbance values of the control and of the test sample, respectively.

The tests were carried out four times. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against extract concentration (100, 80, 50, 30, 20, 10 and 5 mg/l).

2.5.2. FRAP assay

The ferric reducing-antioxidant power (FRAP) assay was conducted according to Benzie and Strain (1996). The principle of the assay is based on the reduction of ferric 2,4,6-tripyridyl-*S*-triazine [Fe(III)-TPTZ] to the ferrous 2,4,6-tripyridyl-*S*-triazine [Fe(II)-TPTZ] complex by a reductant at low pH. This complex has an intense blue colour that can be monitored at 595 nm. The antioxidant efficiency of the samples was calculated with reference to the reaction signal given by an Fe^{2+} solution of known concentration.

Acetate buffer (0.3 M, pH 3.6) was prepared by dissolving 3.1 g $C_2H_3O_2Na \cdot 3H_2O$ and 16 ml of acetic acid in 1 l of distilled water. TPTZ solution was prepared by dissolving 23.4 mg of TPTZ in 7.5 ml of 40 mM HCl solution. Ferric solution (20 mM) was prepared using $FeCl_3 \cdot 6H_2O$. The final working FRAP reagent was prepared freshly by mixing acetate buffer, TPTZ and ferric solutions at a ratio of 10:1:1.

In brief, 900 μl FRAP working reagent was mixed with 90 μl distilled water and was warmed to 37 °C in a water bath. The reagent blank reading was recorded at 595 nm, followed by adding 30 μl of diluted extracts (1:10 v/v) of the same initial concentration (5000 mg/l). The absorbance was taken at 0, 4 and 30 min, against the blank solution. A standard curve was prepared using different concentrations of $FeSO_4 \cdot 7H_2O$ (200–2000 μmol/l). All solutions were freshly prepared. The results were corrected for dilution and expressed in mmol Fe^{2+}/l of plant extract. All determinations were performed four times.

2.6. Total phenolic content of the methanolic extracts

The total phenolic content was determined spectrophotometrically using the Folin-Ciocalteu reagent assay (Singleton & Rossi, 1965), with gallic acid as standard. Briefly, 500 μl of diluted extracts (1:10 v/v) or a standard solution of gallic acid were added to a test tube containing 2.25 ml distilled water. After the addition of 250 μl of Folin-Ciocalteu reagent, the mixture was stirred for 1 min

Table 2
Seasonal variation of the chemical composition of the essential oil from *Pistacia Lentiscus* L., obtained by GC–MS

Compounds	RRI[a]		% Composition[c]			Fit[b]
	CP-Sil 8	DB-Wax	February	May	August	
Tricyclene	930	1006	0.2	0.2	0.2	B
α-Thujene	936	1021	2.0	1.3	0.2	B
α-Pinene	940	1017	17.1[a]	24.9[b]	9.4[c]	A
Camphene	951	1053	1.1	1.1	0,8	B
Thuja-2,4(10)-diene	956	nd	0.1	0.1	tr	B
Sabinene	973	1108	1.0[a]	4.6[b]	6.7[c]	B
β-Pinene	975	1093	2.0[a]	6.9[b]	2.9[a]	A
β-Myrcene	991	1157	0.4	0.3	1.7	A
α-Phellandrene	1001	1206	1.2	1.2	0.9	B
α-Terpinene	1012	1174	2.8	2.2	1.6	A
p-Cymene	1020	1266	7.5[a]	3.5[b]	0.5[c]	A
Limonene	1023	1197	13.9[a]	17.8[b]	9.0[c]	A
(*Z*)-*b*-Ocimene	1035	nd	tr	nd	nd	B
(*E*)-*b*-Ocimene	1046	nd	tr	nd	nd	B
γ-Terpinene	1053	1243	3.6[a]	3.3[b]	3.1[b]	A
α-Terpinolene	1083	1279	1.3[a]	0.9[b]	0.8[b]	B
2-Methylbutyl 2-methylbutanoate	1098	nd	0.1	tr	0.1	B
3-Methylbutyl 3-methylbutanoate	1104	1294	0.3	0.1	0.1	B
2-Methylbutyl 3-methylbutanoate	1106	nd	0.1	0.1	0.1	B
cis-Menth-2-en-1-ol	1115	nd	0.2	0.2	0.2	B
α-Campholenal	1119	nd	0.2	0.2	0.1	B
trans-Pinocarveol	1131	nd	0.2	0.2	0.1	A
cis-β-Terpineol	1139	nd	0.3	0.2	0.2	B
Borneol	1158	nd	0.1	0.1	0.1	A
p-Mentha-1,5-dien-8-ol	1162	nd	0.4	0.1	0.1	B
Terpinen-4-ol	1171	1605	10.6[a]	6.8[b]	10.0[a]	B
α-Terpineol	1185	1704	3.1[a]	2.5[a]	4.0[a]	B
γ-terpineol	1192	nd	0.4	nd	nd	B
Verbenone	1202	nd	0.2	0.1	tr	B
trans-Carveol	1216	nd	0.1	nd	nd	B
Linalyl acetate	1258	1559	tr	tr	tr	A
Bornyl acetate	1283	nd	0.2	0.1	0.2	B
2-Undecanone	1294	nd	0.8	tr	tr	B
α-Cubebene	1347	1457	tr	0.1	0.2	B
α-Terpenyl acetate	1346	nd	0.6	nd	nd	B
Copaene	1372	1490	0.4	0.2	0.7	B
β-Cubebene	1386	1537	tr	0.1	0.3	B
(−)-β-Elemene	1388	1588	0.3	0.2	0.4	B
trans-Caryophyllene	1414	1594	2.1	2.0	4.1	A
α-Humulene	1449	1668	0.8	0.5	1.6	B
trans-Cadina-1(6),4-diene	1471	nd	0.3	0.1	0.2	B
γ-Muurolene	1474	1688	0.9	0.4	1.1	B
Germacrene D	1477	1708	2.7[a]	3.3[a]	13.5[b]	B
β-Selinene	1482	nd	0.3	nd	nd	B
α-Muurolene	1498	nd	0.6	0.3	0.9	B
β-Bisabolene	1498	nd	0.2	nd	nd	B
γ-Cadinene	1512	nd	0.4	0.5	1.5	B
δ-Cadinene	1521	1757	2.5	1.4	3.4	B
trans-Cadina-1(2)-4-diene	1529	nd	0.1	0.1	0.2	B
(−)-Spathulenol	1571	2130	0.3	0.3	0.3	B
Caryophyllene oxide	1576	1984	1.6	1.2	0.5	A
Humulene epoxide II	1588	nd	0.4	0.2	0.2	B
Unknown 1	1590	nd	0.6	nd	nd	C
1-Epi-cubenol	1623	nd	0.4	0.3	1.0	B
Epi-α-cadinol	1637	nd	0.9	0.8	2.6	B
Cadinol isomer	1642	nd	0.5	0.2	0.7	C
α-Cadinol	1650	nd	1.1	1.6	3.8	B
Monoterpene						
Hydrocarbons			54.2[a]	68.3[b]	45[c]	
Oxygenated			18.6[a]	13.3[b]	23.1[a]	

(*continued on next page*)

Table 2 (*continued*)

Compounds	RRI[a]		% Composition[c]			Fit[b]
	CP-Sil 8	DB-Wax	February	May	August	
Sesquiterpene						
Hydrocarbons			11.6[a]	9.2[b]	28.1[c]	
Oxygenated			1.6[a]	1.2[a]	0.5[b]	
Aldehydes			0.2	0.2	0.1	
Ketones			1.6	0.1	tr	
Esters			0.7	0.3	0.5	
Total identified			89.6	92.8	97.5	
Oil yield[d]			0.30	0.30	0.28	

All data represent the mean values of three independent replicates. In each row and for the main chemical compounds and chemical categories, values labeled with different letters were statistically different at $p < 0.05$.

[a] Relative retention indices to C_8–C_{24} *n*-alkanes on DB-Wax and CP-Sil 8 columns.

[b] A: MS data and retention index in agreement with those of authentic compound, B: MS data and retention index in agreement with those in literature, C: MS data in agreement with those in NIST (http://www.nist.gov/srd/nist1a.htm) and WILEY (http://eu.wiley.com/WileyCDA/WileyTitle/productCd-0470047852.html) libraries.

[c] The percentage composition was calculated from the chromatograms obtained on the CP-Sil 8 column. Normalized peak area %, tr: <0.1%, nd: not detected.

[d] v/w %: volume oil to weight of leaves (in g).

and was allowed to stand for 8 min. Then, 2.0 ml of an aqueous solution of Na_2CO_3 (7.5% w/v) were added and the mixture was incubated for 120 min at 25 °C. The absorbance, relative to that of a blank prepared using distilled water, was measured at 760 nm using a double-beam UV–vis spectrophotometer (Jasco V-530). The concentration of total phenolic compounds in the methanolic extracts was determined as mg of gallic acid/g dry plant material by using the regression equation that was obtained from the calibration curve of the gallic acid standard. All determinations were performed four times.

2.7. Statistical analysis

Data obtained from essential oil analysis, methanolic yield, DPPH, FRAP and Folin-Ciocalteu assays were expressed as mean values. All data were analysed with Statistica 6.0 for Windows (StatSoft Inc., Tulsa, OK). Statistical comparisons were made with Student's *t*-test and Fisher's least significant difference test for independent observations. Differences were considered significant at *p* levels of 0.05 and 0.01.

3. Results and discussion

3.1. General

The investigation of the seasonal variation of the essential oil composition of each plant was carried out by GC–MS. The essential oil yields obtained by hydrodistillation are shown in Tables 2 and 3. In order to find a possible commercial exploitation of these wildly grown plants tested, it was decided to examine the methanolic extracts for their potential antioxidant activity (DPPH and FRAP assays) and the total phenolic content (Folin-Ciocalteu assay) for all the periods.

3.2. Composition of essential oils

Tables 2 and 3 list the linear retention indices, percentage composition and yield of the essential oils of *P. lentiscus* L. and *M. communis* L. The yield of the essential oil obtained from the leaves of *P. lentiscus* L., although similar to that in the literature, did not vary across the seasons, as Zrira et al. (2003) reported, whereas significant seasonal variations in the chemical composition were observed.

Fifty seven constituents, which represented 89.6%, 92.8% and 97.5% of the total essential oil of *P. lentiscus* L. for February, May and August respectively, were identified. The essential oil was characterised by a high percentage of monoterpene hydrocarbons (45–68.3%), followed by oxygenated monoterpenes (13.3–23.1%) and sesquiterpene hydrocarbons (9.2–28.1%) (Table 2). The flowering period (May), which differed significantly from the other two periods tested ($p < 0.05$), contained the highest concentration of monoterpene hydrocarbons (68.3%), and the lowest concentration of oxygenated monoterpenes (13.3%) and sesquiterpene hydrocarbons (9.2%). Similar findings have been reported by other authors (Congiu, Falconieri, Marongiu, Piras, & Porcedda, 2002; Zrira et al., 2003). Seasonal variations in the distribution between the monoterpene hydrocarbons and the oxygenated monoterpenes in the plant material could be related to changes throughout the plant's vegetative cycle along with the mild environmental conditions prevailing in the Mediterranean regions during spring.

The opposite was observed during the fruiting period (August). The sesquiterpene hydrocarbon fraction increased whereas the monoterpene hydrocarbon fraction decreased (Table 2). The differences in volatility between these two chemical compound categories along with the high temperatures prevailing during summer could be a

Table 3
Seasonal variation of the chemical composition of the essential oil from *Myrtus communis* L. obtained by GC–MS

Compounds	RRI[a]		% Composition[c]			Fit[b]
	CP-Sil 8	DB-Wax	February	May	August	
Isobutyl isobutyrate	898	1081	2.1[a]	3.0[b]	1.0[c]	C
α-Thujene	936	nd	nd	nd	nd	B
α-Pinene	940	1017	10.9[a]	10.1[a]	11.6[a]	A
Camphene	951	1053	0.1	nd	nd	B
β-Pinene	975	1093	0.1	0.1	0.1	A
δ-3-Carene	nd	1138	nd	nd	nd	B
β-Myrcene	991	1157	0.2	0.3	0.2	A
α-Terpinene	nd	1174	nd	nd	nd	A
p-Cymene	1020	1266	0.3	nd	0.2	A
Limonene	1023	1197	tr	tr	tr	A
1,8-Cineole	1025	1210	13.5[a]	12.7[a]	19.6[b]	A
(*E*)-2-Hexenal	nd	1218	nd	nd	nd	C
(*Z*)-*b*-Ocimene	nd	1234	nd	nd	nd	B
(*E*)-*b*-Ocimene	nd	1250	nd	nd	nd	B
γ-Terpinene	1053	1243	0.1	0.1	0.2	A
cis-Linalool oxide	1067	1448	0.1	0.1	0.0	C
trans-Linalool oxide	nd	1476	nd	nd	nd	B
α-Terpinolene	1083	1279	0.1	0.2	0.2	B
Linalool	1096	1554	7.7[a]	7.0[a]	15.8[b]	A
α-Campholenic acid methyl ester	nd	1582	nd	nd	nd	C
trans-Pinocarveol	1131	1661	0.1	0.2	nd	A
Borneol	1158	1708	0.1	nd	nd	A
Terpinen-4-ol	1171	1605	0.2	0.2	0.2	B
α-Terpineol	1185	1704	1.6[a]	1.8[a]	2.9[b]	B
3-Hexenyl butanoate	nd	1620	nd	nd	nd	C
Myrtenal	nd	1627	nd	nd	nd	C
Myrtenol	1190	1798	2.7[a]	3.5[a]	0.8[b]	A
trans-Geraniol	nd	1856	nd	nd	nd	A
Linalyl acetate	1258	1559	3.6[a]	2.5[a]	6.0[b]	A
trans-Pinocarveyl acetate	1297	1626	0.6	0.7	0.3	B
Myrtenyl acetate	1323	1693	39.0[a]	38.7[a]	23.7[b]	B
p-Menth-1-en-8-ol acetate	1347	nd	0.4	0.4	0.5	B
Neryl acetate	1366	1730	0.4	1.9	3.1	B
Geranyl acetate	1384	1761	1.8	0.4	0.3	B
trans-Caryophyllene	1414	nd	0.3	0.2	0.5	A
α-Humulene	1449	1668	0.5	0.3	0.8	B
Estragole (isoanethole)	nd	1671	nd	nd	nd	B
Unknown 1	1517	2048	3.2[a]	3.6[a]	3.7[a]	C
Caryophyllene oxide	1576	1984	0.5	0.5	0.3	A
Eugenol methyl ether	nd	2016	nd	nd	nd	C
Humulene epoxide II	1588	nd	1.3	1.3	0.7	B
Unknown 2	nd	2293	nd	nd	nd	C
Monoterpene						
Hydrocarbons			11.8[a]	10.8[a]	12.5[a]	
Oxygenated			71.8[a]	70.1[a]	73.2[a]	
Sesquiterpene						
Hydrocarbons			0.8[a]	0.5[a]	1.3[a]	
Oxygenated			0.5[a]	0.5[a]	0.3[a]	
Benzene derivatives			tr	tr	tr	
Total identified			92	90	93	
Oil yield[d]			1.45	1.20	1.45	

All data represent the mean values of three independent replicates. In each row and for the main chemical compounds and chemical categories, the values labeled with different letters were statistically different at $p < 0.05$.

[a] Relative Retention Indices to C_8–C_{24} *n*-alkanes on the DB-Wax and CP-Sil 8.

[b] A: MS data and retention index in agreement with those of authentic compound, B: MS data and retention index in agreement with those in literature, C: MS data in agreement with those in NIST (http://www.nist.gov/srd/nist1a.htm) and WILEY (http://eu.wiley.com/WileyCDA/WileyTitle/productCd-0470047852.html) libraries.

[c] The percentage composition was calculated from the chromatograms obtained on the CP-Sil 8 column. Normalized peak area %, tr: <0.1%, nd: not detected.

[d] v/w %: volume oil to weight of leaves (in g).

reason for this fluctuation in their concentration (Hay & Waterman, 1993).

The major components of *P. lentiscus* L. essential oil were α-pinene (9.4–24.9%) and limonene (9.0–17.8%), while germacrene D (2.7–13.5%), terpinen-4-ol (6.8–10.6%), *p*-cymene (0.5–7.5%), β-pinene (2.0–6.9%), sabinene (1.0–6.7%), γ-terpinene (3.1–3.6%) and α-terpineol (2.5–4.0%) were also present at relatively high percentages. The percentage of most of the individual constituents present in *P. lentiscus* L. essential oil changed significantly during times of harvest. The two major components, along with β-pinene, were found in significantly higher amounts during May (flowering stage) ($p < 0.01$), while *p*-cymene, α-terpinolene and γ-terpinene were found in greater amounts during February (before flowering) ($p < 0.05$). Their percentages decreased as the flowering stage was reached and this can be attributed to the biosynthesis of other compounds, especially oxygenated ones, as *p*-cymene is the main precursor of these compounds. (Nhu-Trang, Casabianca, & Grenier-Loustalot, 2006). The amount of germacrene D increased by four times during August and at the same period sabinene and α-terpineol were also found in high amounts.

The data analysis shows that the chemical profile of our essential oil differs from those of other origins and quantitative differences of individual components exist. For example, Egyptian species was characterised by δ-3-carene, β-bisabolene and β-bourbonene (Pooter, Schamp, Aboutabl, Tohamy, & Doss, 1991), while in the Sardinian species, β-pinene was the major compound, along with β-caryophyllene and β-phellandrene (Congiu et al., 2002). Finally, in Tunisian oil, α-pinene, γ-terpinene and terpinen-4-ol were the main constituents (Douissa et al., 2005). In all cases, differences are directly related to geographical location, while observed variations in the oil chemical composition could also be explained by the existence of chemotypes (Congiu et al., 2002; Zrira et al., 2003).

The yield of the essential oil obtained from the leaves of *M. communis* L. (Table 3), although higher than that reported in the literature, did not vary significantly with seasons, as Jamoussi, Romdhane, Abderraba, Hassine, and Gardi (2005) reported. The essential oil contained 42 compounds accounting for 92%, 90% and 93% of the total oil composition for February, May and August, respectively. It was characterised by a high monoterpene percentage (80.9–85.7%), while sesquiterpenes were found only in low levels (1.0–1.6%). The monoterpene fraction consisted mainly of oxygenated monoterpenes (70.1–73.2%), while the monoterpene hydrocarbons were present in smaller proportions (10.8–12.5%). Although the monoterpene and sesquiterpene fractions reached their highest concentrations during August, they did not vary significantly with season (Table 3).

On the contrary, significant seasonal variations were observed in the individual chemical constituents of *M. communis* L. essential oil. The main compounds identified in this essential oil were myrtenyl acetate (23.7–39.0%), 1,8-cineole (12.7–19.6%), α-pinene (10.1–11.6%) and linalool (7.0–15.8%). The concentrations of myrtenyl acetate, linalool and 1,8-cineole, during August, varied significantly from the other two periods under investigation ($p < 0.05$), whereas α-pinene concentration remained constant. Myrtenyl acetate and myrtenol were in abundance during February and May, whereas the amounts of linalool and linalyl acetate approximately doubled during August.

This chemical profile of our samples, which can be classified in the first chemotype group of myrtenyl acetate/α-pinene proposed by Bradesi et al. (1997), differs from the one reported by Koukos et al. (2001), even though both plants were collected from Greece. According to this report, linalyl acetate was the major constituent (31.4%) followed by a remarkable high concentration of limonene (21.8%). In our samples, the former compound was absent, whereas the latter was detect only in traces. Furthermore, the same authors did not detected either myrtenyl acetate or 1,8-cineole, which were the two major compounds of our samples.

Environmental factors such as geography, temperature, day length, nutrients, etc., were considered to play a key role in the chemical composition of myrtle oil (Scora, 1973). These factors influence the plant's biosynthetic pathways and consequently the relative proportion of the main characteristic compounds. This leads to the existence of different chemotypes which distinguish myrtle oil of different origins, as well as seasonal variations throughout the plant's vegetative cycle (Bradesi et al., 1997; Chalchat et al., 1998; Flamini, Cioni, Morelli, Maccioni, & Baldini, 2004).

3.3. DPPH assay

The results from the DPPH method for methanolic extracts are presented in Table 4. The methanolic extracts of both plants exhibited very good radical-scavenging activities. For *P. lentiscus* L. IC_{50} values ranged between 5.09 and 11.0 mg/l. The IC_{50} values of *M. communis* L. ranged between 9.54 and 17.1 mg/l. The lowest IC_{50} value (highest antioxidant activity) of 5.09 mg/l was obtained for *P. lentiscus* L. harvested during May and varied significantly over the investigated periods ($p < 0.01$). For *M. communis* L., the lowest IC_{50} (9.54 mg/l) was obtained during August, differing significantly from February and May ($p < 0.01$). These values are found to be comparable to those found in *Origanum vulgare* spp. extracts ($IC_{50} = 9.9$ mg/l) (Şahin et al., 2004) and extracts of green tea ($IC_{50} = 4.14$ mg/l) (Takako et al., 1998) and ten times greater than the value found for the reference compound, ascorbic acid ($IC_{50} = 0.51$ mg/l). Both plants, when harvested during February, exhibited the lowest activity (17.1 and 11.0 mg/l, respectively) and their IC_{50} values varied significantly across the seasons ($p < 0.01$).

The ability of the leaf extracts of *P. lentiscus* L. and *M. communis* L. to scavenge free radicals could be attributed

Table 4
Yield, total phenolic content, total antioxidant capacity determined by FRAP assay and IC_{50} values for the methanolic extracts of *Pistacia lentiscus* L. and *Myrtus communis* L. plants

Plant material	Season	Yield[a]	Total phenolics (mg gallic acid/g plant)	FRAP (mmol Fe^{2+}/l)	IC_{50}[b] (mg/l)
Pistacia lentiscus L.	February	45.2 ± 1.2	483 ± 2.7	84.6 ± 3.5	11.0 ± 0.45
	May	61.1[(a)] ± 0.6	588[(a)] ± 32.7	131.4 ± 10.1	5.09 ± 0.10
	August	60.1[(a)] ± 1.0	581[(a)] ± 14.0	105.0 ± 12.2	7.07 ± 0.36
Myrtus communis L.	February	43.4 ± 0.2	307 ± 7.4	63.4 ± 0.4	17.1 ± 0.78
	May	55.0 ± 1.6	352 ± 4.0	65.2 ± 1.2	14.6 ± 0.81
	August	59.5 ± 0.5	373 ± 0.5	70.2 ± 2.3	9.54 ± 0.93

All data represent the mean values of four independent replicates. In each column, for each plant the values were statistically different at $p < 0.05$ between the months, except those labeled with the same letter in parenthesis.
[a] v/w%: volume of methanolic extract to weight of leaves (in g).
[b] IC_{50} value (mg/l) of ascorbic acid: 0.51 ± 0.003.

to an overall participation of their active constituents, such as phenolic acids and flavonoids. Umadevi, Daniel, and Sabnis (1988) Romani, Pinelli, Galardi, Mulinacci, and Tattini (2002) reported that *P. lentiscus* L. is characterised by the presence of phenolic acids (such as gallic acid) and flavonoids (such as myricetin derivatives). A flavan-3-ol (catechin) was also detected in small amounts, also contributing to the antioxidant activity of the extract. A further study, conducted by Baratto et al. (2003) concluded that galloyl derivatives isolated from *P. lentiscus* L. leaves were highly efficient free radical scavengers, whereas quinic acid showed no antioxidant activity. In *M. communis* L. leaves, flavonols (myricetin derivatives) and flavanols (catechin derivatives) were detected in relatively large amounts, with the exception of quercetin derivatives and phenolic acids, which were found only in small amounts (Romani, Pinelli, Mulinacci, Vincieri, & Tattini, 1999; Romani et al., 2004).

Variations in the free radical-scavenging activity during *P. lentiscus* L. and *M. communis* L. development are directly related to the structural characteristics and the amount of phenolic constituents present in plant materials being harvested (Burda & Oleszek, 2001; Rice-Evans, Miller, & Paganga, 1996; Vinson, Dabbagh, Serry, & Jang, 1995c; Wojtaszek, Kruczynski, & Kasprzak, 2002). Wojtaszek et al. (2002) reported that the amount of phenolics is connected with the extent of their accumulation during the vegetative cycle. According to our results, the highest accumulation of phenolic constituents occured at the flowering stage for *P. lentiscus* L. and at the full flowering stage for *M. communis* L. At these stages the highest antioxidant activities were observed. The lowest antioxidant activity of *M. communis* L. extract was observed in February. The same applies for *P. lentiscus* L. extract. Although both plants were in different development stages (Table 1) at this period, they exhibited low antioxidant activity. This fact can be attributed to the limited rate of photosynthesis (Scora, 1973).

3.4. FRAP assay

Total antioxidant capacity (expressed as mmol Fe^{2+}/l plant extract) of methanolic extracts is presented in Table 4. Both plants demonstrated significant antioxidant capacity, with *P. lentiscus* L. methanolic extract exhibiting stronger reducing power (84.6–131.4 mmol Fe^{2+}/l plant extract) to that of *M. communis* L. (63.4–70.2 mmol Fe^{2+}/l plant extract). To our knowledge the FRAP assay was employed for the first time for *P. lentiscus* L.and *M. communis* L. methanolic extracts. The FRAP values of the examined plant extracts are higher than those observed in medicinal plant infusions, as reported by Katalinic, Milos, Kulisic, and Jukic (2006). Although *Melissa folium* exhibited the strongest antioxidant capacity (FRAP value >20 and <30 mmol Fe^{2+}/l plant extract), its FRAP value was five times smaller than that found in our plant extracts. Thus, the total antioxidant capacity, determined as reducing power of extracted *P. lentiscus* L. and *M. communis* L. phenolics, seems to be very promising. The relative activities of well-known natural and synthetic antioxidants have been reported to be close to 2 for vitamin C and (+)-catechin and close to 0.2 for BHT (Benzie & Strain, 1996; Katalinic et al., 2006).

Furthermore, the results of FRAP assay were consistent with those of the DPPH free radical assay for both plants and for all periods examined. The increasing total antioxidant capacity determined for *P. lentiscus* L. and *M. communis* L. leaves during flowering stage (131.4 mmol Fe^{2+}/l) and full flowering stage (70.2 mmol Fe^{2+}/l), respectively, correlated well with the free radical-scavenging activity that was assayed for the same samples and described in the previous paragraph. It seems that seasonal differences observed in the total antioxidant capacity of *P. lentiscus* L. and *M. communis* L. are strongly correlated with their polyphenolic content and particularly with the structure and the concentration of phenolic constituents present in the plant extracts. Various flavonoids, such as quercetin, myricetin, catechin derivatives and phenolic acids (gallic acid) have already been mentioned as constituents of the phenolic profile of *P. lentiscus* L. and *M. communis* L. extracts. This diversity of polyphenols results in different antioxidant capacities. Quercetin, for example, has the stronger FRAP value (4.6), compared to (+)-catechin (2.0) (Katalinic et al., 2006).

3.5. Total phenolic content

The results of total phenolic content, expressed as gallic acid equivalents, and the yield data (v/w %) for the methanolic extracts of *P. lentiscus* L. and *M. communis* L. are presented in Table 4. Our data indicate the possible presence of natural antioxidant phenolic compounds in all methanolic extracts. The percentage extraction yields of *P. lentiscus* L. was found to be four times more than that found in water extracts as reported by Ljubuncic et al. (2005), whereas for *M. communis* L. the yield data were particularly high (Demo, Petrakis, Kefalas, & Boskou, 1998). Furthermore, the determined values were found to be two times greater than the yields from the methanolic extracts of various plant materials, determined by Skerget et al. (2005).

The amount of total phenolics varied in the plant extracts and ranged from 307 to 588 mg gallic acid/g plant material. The highest phenolic concentration was observed in *P. lentiscus* L. extract (588 mg gallic acid/g plant material) harvested during May, and in *M. communis* L. extract (373 mg gallic acid/g plant material) harvested during August. The lowest amounts of phenolic compounds in both plant extracts were observed during February.

As shown in Table 4, the lowest IC_{50} values and the highest FRAP values obtained for *P. lentiscus* L. and *M. communis* L. extracts, in May and August respectively, correspond to a great extent to the highest phenolic content determined at the same periods. Similarly, the same relationship applies for all the other periods examined. This illustrates that a correlation exists between the total phenolic content and the antioxidant properties of the investigated plant extracts. Total phenolic content and total antioxidant capacity of *P. lentiscus* L. and *M. communis* L. extracts showed significant linear correlation ($r = 0.917$, $p < 0.01$). Similarly, a significant linear correlation between total phenolic content and free radical-scavenging activity of both plants was established ($r = -0.907$, $p < 0.01$).

However, further investigation is needed because the Folin-Ciocalteu assay does not differentiate between different phenolic compounds. Substances, such as sugars, aromatic amines, ascorbic acid, sulfur dioxide, iron and other compounds can interfere with the Folin-Ciocalteu assay and correction for interfering substances should be made to measure accurately the phenolic content of the samples (Teow, 2005). Inorganic substances may also interact with Folin-Ciocalteu reagent, giving an inaccurate result (Prior, Wu, & Schaich, 2005).

The structural features of phenolic compounds are also another parameter that should be considered when the Folin-Ciocalteu assay is applied (Frankel, Waterhouse, & Teissedre, 1995). The molar response of the Folin-Ciocalteu method is roughly proportional to the number of phenolic hydroxyl groups in a given substrate, but the reducing capacity is enhanced when two phenolic hydroxyl groups are oriented in *ortho* or *para* positions (Frankel et al., 1995). Since these structural differences are reported to be responsible for antioxidant activity, measurements of phenols in *P. lentiscus* L. and *M. communis* L. extracts may be related to their antioxidant properties (Frankel et al., 1995).

4. Conclusions

This paper deals with the seasonal variation of the essential oil and phenolic content of *P. lentiscus* L. and *M. communis* L. grown in Zakynthos. It should be noted that both plants from this location are examined for the first time.

P. lentiscus L. contains mainly monoterpenes (81.6%) which reach their highest percentage at the flowering stage, during which the strongest antioxidant activity and the highest phenolic content were observed. Concerning *M. communis* L., the monoterpene fraction is the main chemical group of the essential oil in all periods. Its percentage increased slightly to 85.7% during full flowering stage. At this stage,*M. communis* L. also possessed the strongest antioxidant activity and the highest phenolic content. However, myrtenyl acetate, the major constituent of the essential oil of myrtle oil, reached its highest concentration before the flowering stage.

The results of this study further support the view that both *P. lentiscus* L. and *M. communis* L. are promising sources of natural antioxidants. Both plants in all periods tested showed potent antioxidant properties and contained significant amounts of phenolic compounds, as estimated by Folin-Ciocalteu method. These findings confirm the potential uses of *P. lentiscus* L. and *M. communis* L. in food technology and medicine. The present results encourage additional and more in-depth studies on the phenolic composition of the plant extracts and assessment of antioxidant activity of each compound separately. Some phenolic compounds remain to be identified and further biological tests should be conducted.

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References

Adams, R. P. (2001). *Identification of essential oil components by gas chromatography/quadrupole mass spectrometry*. USA: Allured Pub. Corp.

Alamanni, M. C., & Cossu, M. (2004). Radical scavenging activity and antioxidant activity of liquors of myrtle (*Myrtus communis* L.) berries and leaves. *Italian Journal of Food Science, 2*, 197–208.

Baratto, M. C., Tattini, M., Galardi, C., Pinelli, P., Romani, A., Visioli, F., et al. (2003). Antioxidant activity of galloyl quinic derivatives isolated from *Pistacia lentiscus* leaves. *Free Radical Research, 37*, 405–412.

Bentley, R. Y., & Trimen, H. (1980). *Medicinal plants*. London: J. and A Churchill, p. 68.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The frap assay. *Analytical Biochemistry, 239*, 70–76.

Boelens, M. H., & Jimenez, R. (1991). The chemical composition of Spanish Myrtle oils. Part I. *Journal of Essential Oil Research, 3*, 173–177.

Boelens, M. H., & Jimenez, R. (1992). The chemical composition of Spanish Myrtle oils. Part II. *Journal of Essential Oil Research, 4*, 349–353.

Bradesi, P., Tomi, F., Casanova, J., Costa, J., & Bernardini, A. F. (1997). Chemical composition of Myrtle Leaf Essential Oil from Corsica (France). *Journal of Essential Oil Research, 9*, 283–288.

Brand, W., Cuvelier, W., & Berset, M. E. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft Technologie, 28*, 25–30.

Burda, S., & Oleszek, W. (2001). Antioxidant and antiradical activities of flavonoids. *Journal of Agricultural and Food Chemistry, 49*, 2774–2779.

Chalchat, J. C., Garry, R. P., & Michet, A. (1998). Essential oil of Myrtle (*Myrtus Communis* L.) of the Mediterranean Littoral. *Journal of Essential Oil Research, 10*, 613–617.

Congiu, R., Falconieri, D., Marongiu, B., Piras, A., & Porcedda, S. (2002). Extraction and isolation of *Pistacia lentiscus* L. essential oil by supercritical CO_2. *Flavour and Fragrance Journal, 17*, 239–244.

Demo, A., Petrakis, C., Kefalas, P., & Boskou, D. (1998). Nutrient antioxidants in some herbs and Mediterranean plant leaves. *Food Research International, 31*, 351–354.

Douissa, F. B., Hayder, N., Ghedira, L. C., Hammami, M., Ghedira, K., Mariotte, A. M., et al. (2005). New study of the essential oil from leaves of *Pistacia lentiscus* L. (Anacardiaceae) from Tunisia. *Flavour and Fragrance Journal, 20*, 410–414.

Elfellah, M. S., Akhter, M. H., & Khan, M. T. (1984). Anti-hyperglycaemic effect of an extract of *Myrtus communis* in streptozotocin-induced diabetes in mice. *Journal of Ethnopharmacology, 11*, 275–281.

Flamini, G., Cioni, P. L., Morelli, I., Maccioni, S., & Baldini, R. (2004). Phytochemical typologies in some populations of *Myrtus communis* L. on Caprione Promontory (East Liguria, Italy). *Food Chemistry, 85*, 599–604.

Frankel, E. N., Waterhouse, A. L., & Teissedre, P. L. (1995). Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *Journal of Agricultural and Food Chemistry, 43*, 890–894.

Hay, M. K. R., & Waterman, G. P. (1993). *Volatile oil crops: Their biology, biochemistry and production*. New York: Wiley.

Issa, A. Y., Volate, S. R., & Wargovich, M. J. (2006). The role of phytochemicals in inhibition of cancer and inflammation: New directions and perspectives. *Journal of Food Composition and Analysis, 19*, 405–419.

Jamoussi, B., Romdhane, M., Abderraba, A., Hassine, B. B., & Gardi, A. (2005). Effect of harvest time on the yield and composition of Tunisian myrtle oils. *Flavour and Fragrance Journal, 20*, 274–277.

Katalinic, V., Milos, M., Kulisic, T., & Jukic, M. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chemistry, 94*, 550–557.

Koukos, P. K., Papadopoulou, K. I., Papagiannopoulos, A. D., & Patiaka, D. Th. (2001). Chemicals from greek forestry biomass: Constituents of the leaf oil of *Myrtus communis* L. grown in Greece. *Journal of Essential Oil Research, 13*, 245–246.

Lagouri, V., Blekas, G., Tsimidou, M., Kokkini, S., & Boskou, D. (1993). Composition and anti-oxidant activity of essential oils from oregano plants grown wild in Greece. *Zeitschrift für Lebensmitteluntersuchung und-Forschung A, 197*(1), 20–23.

Lagouri, V., & Boskou, D. (1995). Screening for antioxidant activity of essential oils obtained from spices. In G. Charalampous (Ed.). *Food flavors: Generation, analysis and process influence* (Vol. 1, pp. 869–874). Amsterdam: Elsevier.

Ljubuncic, P., Azaizeh, H., Portnaya, I., Coganc, U., & Said, O. (2005). Antioxidant activity and cytotoxicity of eight plants used in traditional Arab medicine in Israel. *Journal of Ethnopharmacology, 99*, 43–47.

Milan, S. (2006). Spice antioxidants isolation and their antiradical activity: A review. *Journal of Food Composition and Analysis, 19*, 531–537.

Montoro, P., Tuberoso, C. I. G., Piacente, S., Perrone, A., De Feo, V., Cabras, P., et al. (2006). Stability and antioxidant activity of polyphenols in extracts of *Myrtus communis* L. berries used for the preparation of myrtle liqueur. *Journal of Pharmaceutical and Biomedical Analysis, 41*(5), 1614–1619.

Nhu-Trang, T. T., Casabianca, H., & Grenier-Loustalot, M. F. (2006). Deuterium/hydrogen ratio analysis of thymol, carvacrol, γ-terpinene and *p*-cymene in thyme, savory and oregano essential oils by gas chromatography–pyrolysis–isotope ratio mass spectrometry. *Journal of Chromatography A, 1132*(1–2), 219–227.

Ozek, T., Demirci, B., & Baser, K. H. C. (2000). Chemical composition of Turkish Myrtle Oil. *Journal of Essential Oil Research, 12*, 541–544.

Pooter, H. L., Schamp, N. M., Aboutabl, E. A., Tohamy, S. F., & Doss, S. L. (1991). Essential oils from the leaves of three *Pistacia* species grown in Egypt. *Flavour and Fragrance Journal, 6*, 229–232.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardised methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*, 4290–4302.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine, 20*, 933–956.

Romani, A., Coinu, R., Carta, S., Pinelli, P., Galardi, C., Vincieri, F., et al. (2004). Evaluation of Antioxidant effect of different extracts of *Myrtus communis* L.. *Free Radical Research, 38*, 97–103.

Romani, A., Pinelli, P., Galardi, N., Mulinacci, N., & Tattini, M. (2002). Identification and quantification of galloyl derivatives, flavonoid glycosides and anthocyanins in leaves of *Pistacia lentiscus* L. *Phytochemical Analysis, 13*, 79–86.

Romani, A., Pinelli, P., Mulinacci, N., Vincieri, F. F., & Tattini, M. (1999). Identification and quantification of polyphenols in leaves of *Myrtus communis*. *Liquid Chromatographia, 49*, 17–20.

Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M., et al. (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chemistry, 91*, 621–632.

Şahin, A. F., Güllüce, M., Daferera, D., Sökmen, A., Sökmen, M., Polissiou, M., et al. (2004). Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. *vulgare* in the Eastern Anatolia region of Turkey. *Food Control, 15*, 557–594.

Scora, R. W. (1973). Essential leaf oil variability in green, variegated and albino foliage of *Myrtus communis*. *Phytochemistry, 12*, 153–155.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology Viticulture, 16*, 144–158.

Skerget, M., Kotnic, P., Hadolin, M., Hras, A. R., Simonic, M., & Knez, Z. (2005). Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chemistry, 89*, 191–198.

Soong, Y. Y., & Barlow, P. J. (2004). Antioxidant activity and phenolic content of selected fruit seeds. *Food Chemistry, 88*, 411–417.

Stocker, P., Yousfi, M., Djerridane, O., Perrier, J., Amziani, R., El Boustani, S., et al. (2004). Effect of flavonoids from various Mediterranean plants on enzymatic activity of intestinal carboxylesterase. *Biochimie, 86*, 919–925.

Takako, Y., Erbo, D., Takako, N., Hiroshi, K., Hitomi, N., Shigeya, T., et al. (1998). In vitro and in vivo studies on the radical-scavenging activity of tea. *Journal of Agricultural and Food Chemistry, 46*, 2143–2150.

Teow, C. (2005). Antioxidant activity and bioactive compounds of sweetpotatoes. Thesis submitted to the Graduate Faculty of North Carolina State University.

Tsimidou, M., & Boskou, D. (1994). Antioxidant activity of essential oils from the plants of the Lamiaceae family. In G. Charalampous

(Ed.), *Spices, herbs and edible fungi* (pp. 273–284). Amsterdam: Elsevier.

Umadevi, I., Daniel, M., & Sabnis, S. D. (1988). Chemotaxonomic studies on some members of Anardiaceae. In *Proceedings of the Indian Academy of Sciences – Plant sciences*, 98(3), pp. 205–208.

Vinson, J. A., Dabbagh, Y. A., Serry, M. M., & Jang, J. (1995c). Plant flavonoids, especially tea flavonols, are powerful antioxidants using an in vitro oxidation model for heart disease. *Journal of Agricultural and Food Chemistry, 43*, 2800–2802.

Wojtaszek, M. E., Kruczynski, Z., & Kasprzak, J. (2002). Variations in the free radical scavenging activity of *Ginkgo biloba* L. leaves in the period of complete development of green leaves to fall of yellow ones. *Food Chemistry, 79*, 79–84.

Young, I. S., & Woodside, J. V. (2001). Antioxidants in health and disease. *Journal of Clinical Pathology, 54*(3), 176–186.

Zrira, S., Elamrani, A., & Benjilali, B. (2003). Chemical composition of the essential oil of *Pistacia lentiscus* L. from Morocco – A seasonal variation. *Flavour and Fragrance Journal, 18*, 475–480.