JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

RP-HPLC Analysis of the Phenolic Compounds of Plant Extracts. Investigation of Their Antioxidant Capacity and Antimicrobial Activity

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Extracts of aromatic plants of Greek origin were examined as potential sources of phenolic compounds. RP-HPLC with UV detection was employed for the identification and quantification of the phenolic antioxidants, present in methanolic extracts. The most abundant phenolic acids were ferulic acid (1.1−280 mg/100 g of dry sample) and caffeic acid (1.2−60 mg/100 g of dry sample). (+)-Catechin and quercetin were the most abundant flavonoids. Apigenin and luteolin were detected in high amounts in *Menta pulegium* and *Thymus vulgaris*, respectively. The antioxidant capacity was determined, in dried ground plants and in their methanol extracts, with the Rancimat test using sunflower oil as substrate. Both pulverized plants and extracts showed antioxidant capacity. Total phenolic content in the extracts was determined spectrometrically according to the Folin−Ciocalteu assay and ranged from 1 to 21 mg of gallic acid/100 g of dry sample. Antimicrobial activity of the extracts against selected microbes was also conducted in this study.

KEYWORDS: Aromatic plants; phenolic antioxidants; RP-HPLC; total phenolics; antimicrobial activity

INTRODUCTION

The importance of aromatic plants as natural antioxidants is well established (*1*). Their main constituents, (poly)phenolic substances, are a class of higher plant secondary metabolites (*2*). They tend to be water soluble, because they frequently occur combined as glycosides, and they are usually located in the cell vacuole (*3*). Polyphenols are antioxidants with redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. They also have metal chelation properties (*4*, *5*). Their significance for the human diet and antimicrobial activity has been recently established (*6*). Indeed, these compounds have been proposed as potential preservatives (*7*), because consumer pressure on the food industry to avoid chemical preservatives has increased over the past decades. With regard to the physiological and pharmacological actions of polyphenols, it was found that these compounds possess molluscicidal, anthelmintic, and antihepatoxic activities. More precisely, it was found that they inhibit human immunodeficiency viral replication (HIV), human simplex virus (HSV), glucosyl transferases of *Streptococcus mutans* (dental carries), ascorbate autoxidation (green tea), cytotoxic effects, tumor promotion, and xanthine, monoamine oxidases (*8*).

Furthermore, the anti-inflammatory, antidiarrheal, antiulcer, antiviral, antiallergic, and vasodilatory actions of these compounds have been also reported (*9*).

Due to the complexity of the natural mixtures of phenolic compounds of various plants it is rather difficult to elucidate their structure and assess the antioxidant and biological potentials. Indeed, the determination of individual flavonoid glycosides from plant materials could prove to be a difficult task (*10*).

The aim of this work was to analyze and identify the major phenolic substances present in some aromatic plants of Greece by using RP-HPLC. This system is a high-resolution chromatographic technique widely used for the simultaneous separation and quantification of phenolic substances. The antioxidant and antimicrobial capacity/activity of these compounds were also studied.

MATERIALS AND METHODS

Standards. Gallic acid, gentisic acid, *p*-coumaric acid, vanillic acid, ferulic acid, syringic acid, (+)-catechin, quercetin, apigenin, naringenin, and eriodictyol were purchased from Sigma-Aldrich (Steinheim, Germany). Luteolin was from Röth (Karlsruhe, Germany). Caffeic acid was from Merck (Darmstadt, Germany). (−)-Epicatechin was from Fluka AG (Buchs, Switzerland). Rutin was from Alexis Biochemicals (Lausen, Switzerland). Hydroxytyrosol, *p*-hydroxybenzoic acid, and butylated hydroxytoluene (BHT) were a kind donation from the National Agricultural Research Foundation (N.AG.RE.F) of Greece. All standards were prepared as stock solutions in methanol. Working standards were made by diluting stock solutions in 62.5% aqueous methanol containing 1 g L^{-1} BHT and 6 M HCl to yield concentrations ranging between 0.5 and 25 mg L^{-1}. Stock/working solutions of the standards were stored in darkness at −18 °C.

Solvents and Reagents. All solvents and reagents from various suppliers were of the highest purity needed for each application. The Folin−Ciocalteu reagent was from Merck.

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10.1021/jf040083t CCC: $30.25
Published on Web 01/20/2005

Plant Material. Dried samples were obtained commercially or collected from different sites in Greece. Some samples were dried in the air (at 25 °C in the dark) and some were lyophilized. All samples were analyzed within 3 months of collection.

Extraction and Hydrolysis. The extraction method used for dried samples was as follows: Forty milliliters of 62.5% aqueous methanol containing BHT (1 g L^{-1}) was added to 0.5 g of dried sample. Then 10 mL of 6 M HCl was added. The mixture was stirred carefully. In each sample nitrogen was bubbled for ~40−60 s. The extraction mixture was then refluxed in a water bath at 90 °C for 2 h. After cooling, it was filtered and made up to 100 mL with methanol (*10*). To prevent enzymic oxidation, extraction of the polyphenols from plants with boiling alcohol is essential and should be adopted routinely (*3*). For the same reason all of this work was carried out in the dark and under a nitrogen atmosphere. Before the determination by HPLC, the samples were filtered quickly through a 0.45 μm membrane filter (Millex-HV).

HPLC Analysis. The analytical HPLC system employed consisted of a JASCO high-performance liquid chromatograph coupled with a UV−vis multiwavelength detector (MD-910 JASCO). The analytical data were evaluated using a JASCO data processing system (DP-L910/V). The separation was achieved on a Waters Spherisorb 5 μm ODS2 4.6 × 250 mm column at ambient temperature. The mobile phase consisted of water with 1% glacial acetic acid (solvent A), water with 6% glacial acetic acid (solvent B), and water/acetonitrile (65:30 v/v) with 5% glacial acetic acid (solvent C). The gradient used was similar to that used for the determination of phenolics in wine (*11*) with some modifications: 100% A, 0−10 min; 100% B, 10−30 min; 90% B/10% C, 30−50 min; 80% B/20% C, 50−60 min; 70% B/30% C, 60−70 min; 100% C, 70−105 min; 100% A, 105−110 min; post-time, 10 min before next injection. The flow rate was 0.5 mL/min, and the injection volume was 20 μL. The monitoring wavelength was 280 nm.

The identification of each compound was based on a combination of retention time and spectral matching.

Antioxidant Capacity (Rancimat Test). Samples of sunflower oil (3.5 g) containing 0.02% w/w extract or 2% w/w ground material were subjected to oxidation at 110 °C (air flow = 20 L/h). The standard compounds (0.02% addition) were also examined. Induction periods, IP (hours), were recorded automatically. The coefficient of variation (CV, %) of the method was 3.4 (n = 3). The protection factors (PF) were calculated according to the following formula: ($PF = IP_{extract}/IP_{control}$) (*12*).

Determination of Total Phenolic Content of Plant Extracts. Total phenolic content was measured according to the Folin−Ciocalteu assay (*4*). Results were expressed as milligrams of gallic acid per gram of dry sample.

Microbial Strains. The methanolic extracts were tested against a panel of pathogenic microorganisms, including *Escherichia coli* 0157:H7 NCTC 12900, *Salmonella enteritidis* PT4, *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ScottA, and *Bacillus cereus.* Microorganisms were stored frozen in bead vials (Protect; Technical Service Consultants Ltd., Heywood, Lancashire, U.K.). Resuscitation of bacterial strains was carried out in 10 mL of BH broth (Merck catalog no. 1.10493) incubated at 37 °C overnight for *E. coli* and *S. enteritidis* PT4, at 35 °C for *B. cereus*, and at 30 °C for *L. monocytogenes*.

Antimicrobial Assay. Resuscitated cultures were diluted 10-fold in Ringer's solution (LabM) for the inoculation of 10 mL of BH broth (Merck catalog no. 1.10493) to give an initial suspension of ~10−100 cfu/mL. All broths then were incubated statically at 37 °C for *E. coli* 0157:H7 NCTC 12900 and *S. enteritidis* PT4, at 35 °C for *B. cereus*, and at 30 °C for *L. monocytogenes* ScottA and *S. aureus* ATCC 6538 for 18−24 h to ensure that all microorganisms were well into the stationary phase when tested. Susceptibility of the test organism to the extract was determined by employing the standard disk diffusion technique. The bacterial suspensions were diluted 10-fold in Ringer's solution (LabM), and 0.1 mL from the appropriate dilution was spread plated on BH agar (Merck catalog no. 1.13825) in order to give a population of ~10^6 colony-forming units (cfu)/plate. Sterile paper disks with a diameter of 6.48 mm (Whatman no. 2) were placed onto the inoculated agar surface. Five microliters of each plant extract was added to the paper disks. Each experiment was carried out in triplicate. Petries were incubated for 48 h, at 37 °C for *E. coli* 0157:H7 NCTC 12900 and *S. enteritidis* PT4, at 35 °C for *B. cereus*, and at 30 °C for *L. monocytogenes* ScottA and *S. aureus* ATCC 6538. After incubation, the inhibition zones were estimated by taking photos of Petries with a Sony camera (x-wave HAD SSC-DC50AP) and processed using Impuls Vision XL 2.5 software. Each inhibition zone diameter was measured three times and the average taken.

RESULTS AND DISCUSSION

Antioxidant Capacity. The antioxidant capacity (expressed as PF values) and the total phenolic content of all extracts are shown in **Table 1**. The amount of total phenolics varied slightly in plant materials and ranged from 1.2 to 21 mg of gallic acid/g of dry sample. The highest amount was found in rosemary (*Rosmarinus officinalis*), and the lowest in *Tilia argentea*. Similar amounts in plant phenolics from herbs and medicinal plants collected in Finland have been reported (*13*).

The outcome of the Rancimat test supports the hypothesis that aromatic plants are good sources of natural antioxidants such as the phenolic compounds. When working accurately, this method offers an efficient, simple, and automated measuring principle. Chain-breaking antioxidants react with peroxyl radicals, introducing a lag period into the peroxidation process that is equal with the time taken for the antioxidant to be consumed (*14*). When ground material was added to sunflower oil, protection factors were slightly higher compared to the addition of methanol extracts, with the exception of *Cassia artemisioides* and *Malva silvestris.* Extracts of *T. argentea*, *Lagoecia cuminoides*, and *Crocus sativus* had shown prooxidative effect. The PF values for the standard compounds (0.02% addition) examined ranged from 1.2 to 1.5 for all phenolic acids but gallic acid, which had PF = 4.5. (+)-Catechin, hydrated, and (−)-epicatechin had PF values of 1.8 and 2.5, respectively, whereas the flavonoids (rutin, quercetin, apigenin, luteolin, eriodictyol, and naringenin) had PF values ranging from 1 to 1.2. The PF value for *p*-hydroxytyrosol was 1.4 and that for BHT was 1.8. These values explain the antioxidant potential of the examined plant extracts and depend on the total phenol content, without being proportional. Similar PF values for ethanol and acetone extracts of plants of Greek origin have been reported (*12*). Because of the complex nature of the mixture of phenolic compounds present in aromatic plants and because of their instability under different conditions and in the presence of air, it is difficult to elucidate their structure. Methods for their quantification are partly successful. A wide range of color reactions have been used to determine total polyphenols, including the reaction with the Folin−Ciocalteu reagent. However, as each phenolic compound gives a different color, the results of these determinations are of limited value. The most promising approach is the quantitative determination of the substances by HPLC.

HPLC Analysis. RP-HPLC with C_{18} columns is the most popular technique for the analysis of polyphenols of different foods, despite the fact that the separation of procyanidins is not satisfactory (*15*). A UV−vis multiwavelength detector was used because all phenolic compounds show intense absorption in the UV region of the spectrum. The present method is simple, easy to use, and effective enough for the identification and quantification of major phenolic compounds in aromatic plants. A similar technique has been reported by other authors for the analysis of major flavonoid aglycons (*10*, *16*). A typical HPLC profile of the phenolic constituents of an analyzed plant is presented in **Figure 1**.

Spherisorb ODS2 stationary phase, which was used in this study to separate phenolic acids and flavonoids of aromatic

Table 1. Total Phenolics in Plant Extracts and Their Antioxidant Capacity (Expressed as PF Values)

Latin name	part examined	drying method[a]	total phenolics[b] (mg of gallic acid/g of dry sample)	PF[c,d] (ground material)	PF (methanol extracts)
Caprifoliaceae					
Sambucus migra	flower	air	13.8 ± 0.4	1.3	1.2
Umbelliferae					
Anethum graveolens	herb	air	12.5 ± 0.3	1.4	1.2
Coriandrum sativum	leaves	air	5.2 ± 0.2	1.2	1.1
Petroselinum sativum	leaves	air	9.6 ± 0.4	1.6	1.4
Pimpinella anisum	herb	air	1.8 ± 0.1	1.2	1.2
Iridaceae					
Crocus sativus	leaves	f/v	6.6 ± 0.2	1	0.8
Compositae					
Taraxacum officinale	leaves	f/v	5.4 ± 0.3	1.8	1.7
Artemisia arborense	herb	air	11.2 ± 0.5	1.4	1.1
Matricaria chamomilla	flower	air	8.5 ± 0.3	1.4	1.3
Tiliaceae					
Tilia argentea	leaves	air	1.2 ± 0.1	1.1	0.9
Leguminosae					
Cassia artemisioides	herb	air	2.9 ± 0.1	1.3	1.4
Malvaceae					
Malva silvestris	herb	f/v	4.5 ± 0.2	1.4	1.5
Labiateae					
Salvia officinalis	leaves	air	13.6 ± 0.4	1.4	1.2
Thymus vulgaris	herb	air	19.2 ± 0.3	4.7	4.1
Hyssopus officinalis	herb	air	5.2 ± 0.2	1.3	1.1
Menta viridis	leaves	air	16.5 ± 0.3	1.4	1.3
Rosmarinus officinalis	leaves	air	21 ± 0.5	5.1	4.5
Origanum majorana	herb	air	16.9 ± 0.3	2.1	1.8
Lavandula vera	flower	air	7.3 ± 0.2	1.3	1.2
Menta pulegium	leaves	air	8.4 ± 0.1	1.6	1.4
Mellisa officinalis	leaves	air	17 ± 0.6	1.4	1.2
Ocynum basilicum	leaves	air	7.4 ± 0.3	1.4	1.1
Lagoecia cuminoides	herb	air	3.1 ± 0.2	1.2	0.9
Teucrium chamedris	herb	air	9 ± 0.2	1.4	1.1
Apiaceae					
Lagoecia cuminoides	fruit	f/v	1.9 ± 0.1	1.2	1
Rubiaceae					
Asperoulla odorata	leaves	f/v	14.3 ± 0.4	1.7	1.6
Cyperaceae					
Cyperus rotundus	leaves	f/v	4.2 ± 0.2	1.5	1.3
Apocynaceae					
Vinca rosea	leaves	f/v	15.9 ± 0.2	1.8	1.5

[a] Air, air-drying; f/v, freeze vacuum, i.e., lyophilization. [b] Mean of duplicate assays. [c] PF, protection factor. [d] CV (%) = 3.4, $n = 3$.

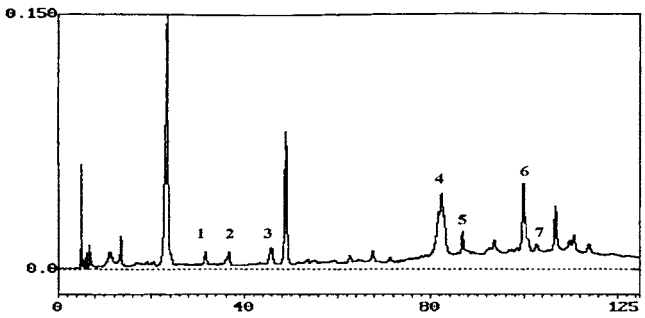

Figure 1. Typical HPLC chromatogram of *Menta pulegium* where: (1) hydroxytyrosol, (2) (+)-catechin, (3), caffeic acid, (4) ferulic acid, (5) naringenin, (6) apigenin, and (7) luteolin.

plants, produced satisfactory results. After extraction and acid hydrolysis, the content of phenolic substances was determined. The amount of phenolic acids detected in the analyzed samples is shown in **Table 2**. Additionally, the content of flavonoids identified in the same plant extracts is shown in **Table 3**. Results are expressed in milligrams per 100 g of dry sample.

Another phenolic compound that was detected as well in some samples was hydroxytyrosol. *Vinca rosea* contained large amounts of this compound (310 mg/100 g of dry sample), whereas *Menta pulegium* contained a moderate amount (21 mg/100 g of dry sample). In most of the other plant extracts hydroxytyrosol was detected in traces. The most abundant phenolic acids were ferulic acid (1.1−280 mg/100 g of dry sample) and caffeic acid (1.2−60 mg/100 g of dry sample). *M. pulegium* contained the highest amount of ferulic acid (280 mg/100 g of dry sample). *p*-Coumaric acid was detected in only *T. argentea*, *T. vulgaris*, and *Cyperus rotundus*. (+)-Catechin and quercetin were the most abundant flavonoids.

Apigenin and luteolin were detected in high amounts in *M. pulegium* and *T. vulgaris*, respectively. Rutin (quercetin 3-*O*-rhamnose glycoside) was detected only in *Menta viridis* and *Asperoulla odorata*. The absence of rutin can be attributed to the fact that rutin was hydrolyzed to quercetin (aglycon).

Naringenin was detected only in *Lavandula vera* and *M. pulegium*. Another flavanone, eriodictyol, was also detected in other plant extracts (**Table 3**). Papers about most of the examined plant extracts are very scarce in the literature. Optimization of acidic conditions for the hydrolysis of flavonoid glycosides in a range of fruits, vegetables, and beverages has been described by Hertog et al. (*17*). Antioxidant activities of polyphenols from sage (*Salvia officinalis*) have been reported (*18*). Phenolic compounds such as vanillic acid, caffeic acid, luteolin, and apigenin in certain herbs such as rosemary, thyme, sage, and basil were found in concentrations similar to those reported by other researchers (*13*). The flavonol quercetin and

Table 2. Content of Phenolic Acids in 27 Aromatic Plant Extracts

	content[a] (mg/100 g of dry sample)							
plant	gallic acid	gentisic acid	caffeic acid	*p*-coumaric acid	vanillic acid	syringic acid	ferulic acid	*p*-hydroxybenzoic acid
Sambucus migra	ND	1.5	37.5	ND	0.8 ± 0.02	ND	39.8 ± 0.06	ND
Anethum graveolens	ND	ND	ND	ND	1.6 ± 0.01	ND	ND	ND
Crocus sativus	1.2 ± 0.02	ND	ND	ND	ND	ND	ND	ND
Coriandrum sativum	ND	ND	ND	ND	0.6 ± 0.01	ND	ND	ND
Taraxacum officinale	ND	ND	3 ± 0.02	ND	ND	ND	2 ± 0.04	ND
Rosmarinus officinalis	ND	3.2 ± 0.03	2	ND	2 ± 0.02	2 ± 0.01	5.2 ± 0.03	1.4 ± 0.04
Petroselinum sativum	0.7 ± 0.02	1.6 ± 0.01	ND	0.4 ± 0.01	0.6 ± 0.02	ND	ND	1.3 ± 0.02
Artemisia arborense	1.1 ± 0.02	ND	38.4 ± 0.06	ND	1.3 ± 0.02	ND	30.8 ± 0.07	ND
Tilia argentea	ND	ND	ND	1.2 ± 0.01	0.5 ± 0.02	ND	ND	ND
Cassia artemisioid	ND	ND	ND	ND	ND	ND	ND	1.5 ± 0.02
Salvia officinalis	ND	2.4 ± 0.03	ND	ND	ND	ND	4.9 ± 0.0	1.2 ± 0.01
Matricaria chamomilla	ND	ND	1.2 ± 0.02	ND	ND	ND	1.6 ± 0.01	2 ± 0.02
Thymus vulgaris	ND	2.8 ± 0.02	5.8 ± 0.04	1.2 ± 0.01	ND	5 ± 0.03	ND	1.4 ± 0.01
Hyssopus officinalis	ND	1.2 ± 0.01	6.5 ± 0.02	ND	ND	3 ± 0.02	13.2 ± 0.04	1.2 ± 0.01
Malva silvestris	1.1 ± 0.01	ND	ND	ND	ND	ND	4.3 ± 0.02	1.4 ± 0.01
Menta viridis	0.9 ± 0.02	ND	6 ± 0.02	ND	0.7 ± 0.02	ND	5.6 ± 0.03	ND
Pimpinella anisum	1.1 ± 0.01	ND	0.8 ± 0.02	ND	ND	ND	1.2 ± 0.01	ND
Origanum majorana	ND	1.4 ± 0.02	4.2 ± 0.03	ND	ND	ND	9.2 ± 0.04	1.2 ± 0.01
Lavandula vera	0.5 ± 0.01	3.1 ± 0.02	0.4 ± 0.02	ND	0.6 ± 0.02	ND	1.3 ± 0.01	1.6 ± 0.02
Menta pulegium	ND	ND	60 ± 0.08	ND	0.8 ± 0.02	ND	280 ± 0.08	ND
Mellisa officinalis	ND	2.1 ± 0.02	13.8 ± 0.04	ND	ND	ND	48 ± 0.05	2.3 ± 0.02
Ocynum basilicum	ND	1.5 ± 0.02	2.8 ± 0.01	ND	ND	ND	4 ± 0.02	1.3 ± 0.01
Lagoecia cuminoides	1 ± 0.01	ND	6.6 ± 0.02	ND	1.3 ± 0.01	ND	4.2 ± 0.02	ND
Asperoulla odorata	1.2 ± 0.01	ND	34.6 ± 0.06	ND	ND	ND	84 ± 0.07	ND
Cyperus rotundus	1.4 ± 0.01	ND	ND	3.8 ± 0.03	ND	ND	ND	ND
Vinca rosea	42 ± 0.03	ND	ND	ND	1.3 ± 0.01	ND	250 ± 0.08	ND
Teucrium chamedris	0.6 ± 0.02	ND	0.7 ± 0.03	ND	ND	ND	1.1 ± 0.01	ND

[a] Each value is the mean (mg/100 g of dry sample) of two replications ± standard deviation; ND, not detected.

Table 3. Flavonoid Content in 27 Aromatic Plant Extracts

	content[a] (mg/100 g of dry sample)							
sample	quercetin, hydrated	apigenin	luteolin	naringenin	eriodictyol	rutin	(+)-catechin, hydrated	(–)-epicatechin
Sambucus migra	ND	ND	ND	0.6 ± 0.02	ND	ND	ND	ND
Anethum graveolens	36 ± 0.06	ND	ND	ND	ND	ND	ND	4.5 ± 0.04
Crocus sativus	ND	ND	ND	ND	0.3 ± 0.02	ND	ND	ND
Coriandrum sativum	3 ± 0.02	ND	ND	ND	ND	ND	ND	ND
Taraxacum officinale	ND	ND	ND	ND	ND	ND	ND	0.4 ± 0.02
Rosmarinus officinalis	ND	ND	1.6 ± 0.01	ND	ND	ND	1.5 ± 0.02	ND
Petroselinum sativum	1.3 ± 0.03	60 ± 0.07	2.1 ± 0.03	ND	ND	ND	ND	ND
Artemisia arborense	ND	ND	ND	ND	ND	ND	ND	ND
Tilia argentea	ND	ND	ND	ND	ND	ND	ND	ND
Cassia artemisioides	2 ± 0.02	ND	ND	ND	0.1 ± 0.01	ND	ND	ND
Salvia officinalis	ND	0.3 ± 0.02	0.2 ± 0.02	ND	ND	ND	2.5 ± 0.02	ND
Matricaria chamomilla	ND	ND	ND	ND	ND	ND	ND	ND
Thymus vulgaris	ND	4 ± 0.01	36 ± 0.06	ND	ND	ND	ND	0.3 ± 0.02
Hyssopus officinalis	ND	1.2 ± 0.02	ND	ND	ND	ND	5.7 ± 0.03	ND
Malva silvestris	ND	ND	ND	ND	ND	ND	ND	ND
Menta viridis	ND	0.7 ± 0.02	ND	ND	ND	1.4 ± 0.01	2 ± 0.02	ND
Pimpinella anisum	ND	ND	ND	ND	ND	ND	ND	ND
Origanum majorana	ND	0.8 ± 0.01	ND	ND	ND	ND	2.6 ± 0.02	ND
Lavandula vera	ND	ND	ND	3.3 ± 0.02	ND	ND	2.4 ± 0.03	ND
Menta pulegium	ND	110 ± 0.07	15	42 ± 0.06	ND	ND	26 ± 0.05	ND
Mellisa officinalis	ND	ND	ND	ND	1.1 ± 0.01	ND	21 ± 0.04	ND
Ocynum basilicum	ND	ND	ND	ND	ND	ND	1.2 ± 0.02	ND
Lagoecia cuminoides	ND	ND	ND	ND	0.5 ± 0.02	ND	ND	ND
Asperoulla odorata	ND	ND	ND	0.9 ± 0.02	4 ± 0.02	1.5 ± 0.01	0.6 ± 0.03	1.3 ± 0.03
Cyperus rotundus	ND	ND	ND	ND	ND	ND	ND	0.3 ± 0.01
Vinca rosea	ND	ND	ND	ND	ND	ND	ND	ND
Teucrium chamedris	0.2 ± 0.01	0.9 ± 0.02	1.2 ± 0.02	ND	ND	ND	ND	ND

[a] Each value is the mean (mg/100 g of dry sample) of two replications ± standard deviation; ND, not detected.

the two major flavones luteolin and apigenin have been identified and simultaneously quantified in 62 types of edible tropical plants in Malaysia (*19*).

Phenolic compounds are found usually in nature as esters and rarely as glycosides or in free form (*20*). Thus, hydrolysis was needed for their identification and quantitative determina-

Table 4. Antimicrobial Activity of Plant Extracts; Sample Amount of 5 μL ($n = 3$)

plant extract	*E. coli* 0157:H7 NCTC12900	*S. enteritidis* PT4	*S. aureus* ATCC 6538	*L. monocytogenes* ScottA	*B. cereus*
S. migra	–[a]	–	–	–	–
A. graveolens	–	–	–	–	–
C. sativus	–	–	–	–	–
C. sativum	–	–	–	–	–
T. officinale	~[b]	–	–	–	–
R. officinalis	~	–	–	–	~
P. sativum	~	–	–	–	–
A. arborense	–	–	–	–	–
T. argentea	–	–	–	–	~
C. artemisioides	–	–	–	–	++[d]
S. officinalis	–	–	–	~	+[c]
M. chamomilla	–	~	–	–	~
T. vulgaris	~	–	–	~	–
H. officinalis	~	–	–	~	~
M. silvestris	~	–	–	~	~
M. viridis	–	~	–	–	–
.P. anisum	~	–	–	–	~
O. majorana	~	–	–	–	–
L. vera	~	–	–	–	~
M. pulegium	~	–	~	–	~
M. officinalis	~	–	–	–	~
O. basilicum	~	–	~	~	~
L. cuminoides	~	–	~	~	~
A. odorata	~	–	–	+	~
C. rotundus	~	–	–	~	~
V. rosea	~	–	~	++	~
T. chamedris	~	–	~	++	

[a] –, no antimicrobial capacity, i.z. of sample < i.z. of solvent (62.5% aqueous methanol). [b] ~, slight antimicrobial capacity, i.z. of sample 1–3 mm > i.z. of solvent. [c] +, moderate antimicrobial capacity, i.z. of sample 3–4 mm > i.z. of solvent. [d] ++, clear antimicrobial capacity, i.z. of sample 4–10 mm > i.z. of solvent.

tion. Flavonoids are also present in plants in the form of glycosides. Any flavonoid may occur in a plant in several glycosidic combinations. For this reason, hydrolysis was used to release the aglycons, which can be further investigated by HPLC. The isolation and quantification of phenolic compounds in plant material are difficult because of their chemical complexity. Papers about most of the examined plant extracts are very scarce in the literature. The data presented in **Tables 1−3** are considered to be indicative of the phenolic content of these aromatic plants. Time of harvest and storage conditions are considered to be responsible for the observed variations in the phenolic contents.

Antimicrobial Activity. Antimicrobial assays described in the literature include measurement of (i) the radius or diameter of the zone of inhibition of bacterial growth around paper disks impregnated with (or wells containing) an antimicrobial compound on agar media; (ii) the inhibition of bacterial growth on an agar medium with the antimicrobial compound diffused in the agar; (iii) the minimum inhibitory concentration (MIC) of the antimicrobial compound in liquid media; and (iv) the changes in optical density or impedance in a liquid growth medium containing the antimicrobial compound (*21*). To screen the antimicrobial activity of "unknown" compounds, the methodology ii is considered to be the simplest, by which the results are obtained rapidly. For example, Vardar-Unlu et al. (*21*) and Thangadurai et al. (*22*) have used the disk diffusion method to determine the antimicrobial activity of essential oil and plant extracts using several microbial strains.

The antimicrobial activities of plant extracts are shown in **Table 4**. The plant extracts found to be more effective in inhibiting the microorganisms tested were *Asperoulla odorata* and *Vinca rosea. Cassia artemisioides* and *Salvia officinalis* had clear and moderate effects on *B. cereus*, respectively. *L. monocytogenes* ScottA and *B. cereus* were the most sensitive microorganisms to the plant extracts examined in this study. On the contrary, almost all of the extracts failed to inhibit *S. enteritidis* PT4. The results in **Table 4** reveal that Gram-positive bacteria are more sensitive to the plant extracts than Gram-negative bacteria (*E. coli* 0157:H7 NCTC12900, *S. enteritidis* PT4). It should be taken into account that the relatively low inhibition found in this study could be influenced by the fact that the inhibition area depends on the ability of the antimicrobial compound to diffuse uniformly through the agar. Thus, a greater inhibition can be revealed if alternative methodology can applied.

ABBREVIATIONS USED

RP-HPLC, reversed phase high-performance liquid chromatography; UV−vis, ultraviolet−visible; HIV, human immunodeficiency viral replication; HSV, human simplex virus; BHT, butylated hydroxytoluene; IP, induction period; CV, coefficient of variation; PF, protection factor; MIC, minimum inhibitory concentration.

ACKNOWLEDGMENT

We thank Dr. Athanasios Malouxos for valuable criticism and constructive comments, especially about HPLC determinations.

LITERATURE CITED

(1) Dapkevicious, A.; Venskutonis, R.; Van Beek, T. A.; Linssen, J. P. H. Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. *J. Sci. Food Agric.* **1998**, *77*, 140−146.

(2) Dillard, C. J.; German, J. B. Phytochemicals: nutraceuticals and human health. *J. Sci. Food Agric.* **2000**, *80*, 1744−1756.

(3) Harborne, J. B. Phenolic compounds. In *Phytochemical Methods, a Guide to Modern Techniques of Plant Analysis*, 3rd ed.; Chapman and Hall: London, U.K., 1998; pp 40−106.

(4) Kähkönen, M. P.; Hopia, A. I.; Heikki, J. V.; Rauha, J.-P.; Pihlaja, K.; Kujala, T. S.; Heinonen, M. Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.* **1999**, *47*, 3954−3962.

(5) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* **1997**, *2*, 152−159.

(6) Rauha, J.-P.; Remes, S.; Heinonen, M.; Hopia, A.; Kähkönen, M.; Kujala, T.; Pihlaja, K.; Vuorela, H.; Vuorela, P. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *Int. J. Food Microbiol.* **2000**, *56*, 3−12.

(7) Nychas, G.-J. E.; Tassou, C. C.; Skandamis, P. Making the most of herbs, spices and their active components. In *Natural Antimicrobials for the Minimal Processing of Foods*; Roller, S., Ed.; Woodhead Publishing: Cambridge, U.K., 2003; pp 176−200.

(8) Haslam, E. Polyphenols and herbal medicines. In *Practical Polyphenols, from Structure to Molecular Recognition and Physiological Action*; Cambridge University Press: Cambridge, U.K., 1998; pp 298−334.

(9) Bravo, L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr. Rev.* **1998**, *56*, 317−333.

(10) 0) Mattila, P.; Astola, J.; Kumpulainen, J. Determination of flavonoids in plant material by HPLC with diode-array and electro-array detections. *J. Agric. Food Chem.* **2000**, *48*, 5834−5841.

(11) Parrilla, M. C. G.; Heredia, J. F.; Troncoso, M. A. Sherry wine vinegars: phenolic composition changes during aging. *Food Res. Int.* **1999**, *32*, 433−440.

(12) Exarchou, V.; Nenadis, N.; Tsimidou, M.; Gerothanasis, I. P.; Troganis, A.; Boskou, D. Antioxidant activities and phenolic composition of extracts from greek oregano, greek sage, and summer savory. *J. Agric. Food Chem.* **2002**, *50*, 5294−5299.
(13) Zheng, W.; Wang, S. Y. Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food Chem.* **2001**, *49*, 5165−5170.
(14) Aruoma, O. I.; Spencer, J. P. E.; Rossi, R.; Aeschbach, R.; Khan, A.; Mahmood, N.; Munoz, A.; Murcia, A.; Butler, J.; Halliwell, B. An evaluation of the antioxidant and antiviral action of extracts of rosemary and provencal herbs. *Food Chem. Toxicol.* **1996**, *34*, 449−456.
(15) Suarez, B.; Picinelli, A.; Mangas, J. J. Solid-phase extraction and high-performance liquid chromatographic determination of polyphenols in apple musts and ciders. *J. Chromatogr. A* **1996**, *727*, 203−209.
(16) Justesen, U.; Knuthsen, P. Composition of flavonoids in fresh herbs and calculation of flavonoid intake by use of herbs in traditional Danish dishes. *Food Chem.* **2001**, *73*, 245−250.
(17) Hertog, M. G. L.; Hollman, P. C. H.; Venema, D. P. Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *J. Agric. Food Chem.* **1992**, *40*, 1591−1598.
(18) Lu, Y.; Foo, L. Y. Antioxidant activities of polyphenols from sage (*Salvia officinalis*). *Food Chem.* **2001**, *75*, 197−202.
(19) Miean, K. H.; Suhaila, M. Flavonoid (myricetin, quercetin, kaempherol, luteolin, and apigenin) content of edible tropical plants. *J. Agric. Food Chem.* **2001**, *49*, 3106−3112.
(20) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933−956.
(21) Vardar-Ünlü, G.; Candan, F.; Sökmen, A.; Daferera, D.; Polissiou, M.; Sökmen, M.; Dömnez, E.; Tepe, B. Antimicrobial and antioxidant activity of the essential oil and methanol extracts of *Thymus pectinatus* fisch. et mey. var. *pectinatus* (Lamiaceae). *J. Agric. Food Chem.* **2003**, *51*, 63−67.
(22) Thangadurai, D.; Anitha, S.; Pullaiah, T.; Reddy, P. N.; Ramachandraiah, O. S. Essential oil constituents and in vitro antimicrobial activity of *Decalepis hamiltonii* roots against foodbome pathogens. *J. Agric. Food Chem.* **2002**, *50*, 3147−3149.

Received for review February 23, 2004. Accepted October 16, 2004.

JF040083T