Comparative Analysis of Flavonoid Profile, Antioxidant and Antimicrobial Activity of the Berries of *Juniperus communis* L. var. *communis* and *Juniperus communis* L. var. *saxatilis* Pall. from Turkey

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The present study was designed to define and compare the flavonoid composition and the biological potential of berries methanol extracts of *Juniperus communis* L. var. *communis* (Jcc) and *Juniperus communis* L. var. *saxatilis.* Pall. (Jcs) from Turkey. Total polyphenols (Folin−Ciocalteau method) were 3-fold higher in Jcc (59.17 ± 1.65 mg GAE/g extract) than in Jcs (17.64 ± 0.09 mg GAE/g extract). Flavonoid and biflavonoid content, evaluated by HPLC-DAD−ESI-MS analysis, was higher in Jcc (25947 ± 0.86 and 4346 ± 3.95 μg/g extract) than in Jcs (5387 ± 34.88 and 1944 ± 26.88 μg/g extract). The HPLC analysis of Jcc allowed the separation of 16 flavonoids; hypolaetin-7-pentoside and quercetin-hexoside are the main compounds. Moreover, gossypetin-hexoside-pentoside and gossypetin-hexoside were identified for the first time in Jcc berries. In Jcs eight flavonoids were identified: quercetin-hexoside and isoscutellarein-8-*O*-hexoside are the most abundant compounds. The in vitro antioxidant activity was determined using different methods; Jcc was found to be more active than Jcs in the DPPH test (IC$_{50}$ of 0.63 ± 0.09 mg/mL and 1.84 ± 0.10 mg/mL) in reducing power assay (12.82 ± 0.10 ASE/mL and 64.14 ± 1.20 ASE/mL), and in TBA assay (IC$_{50}$ of 4.44 ± 0.70 μg/mL and 120.07 ± 3.60 μg/mL). By contrast, Jcs exhibited more elevated Fe^{2+} chelating ability than Jcc. The extracts were also studied for their antimicrobial potential, displaying antimicrobial capacity only against Gram-positive bacteria.

KEYWORDS: *Juniperus communis* var. *communis*; *Juniperus communis* var. *saxatilis*; HPLC-DAD−ESI-MS; flavonoids; antioxidant activity; antimicrobial activity

INTRODUCTION

Juniperus communis L. (Cupressaceae) is an evergreen shrub or tree diffused in Europe, Asia and North America; it grows in scrubs, pastures, cliffs, from the mountains to the sea coastal. *J. communis* L. is a dioecious species, with flowers gathered in axillary aments; false fruits, cones (improperly called "berries") ripening the second or third year, are green the first year, then bluish-black or sometimes purplish-red when fully ripe (*1−3*). According to the Flora Europaea, *J. communis* L. includes three subspecies: *communis*, *nana* Willd., and *hemisphaerica* (Presl) Nyman. *J. communis* L. ssp. *nana* (Willd) Syme (syn. *J. communis* var. *saxatilis* Pall.) is considered by some botanists to have resulted by adaptation to cold climates of *J. communis* L. ssp. *communis*; others may consider it as a distinct subspecies (*1, 2*). Although they can be distinguished by morphological features,

there are no clear genetic differences between the subspecies *communis* and *nana* so that some authors tend to relegate the subspecies as varieties (*2, 4*); in fact, two varieties were accepted in the "Flora of Turkey" for *J. communis* as var. *communis* and var. *saxatilis* (*3*).

Dried ripe cone berry of *J. communis* (Iuniperi pseudo-fructus) is registered in the European Pharmacopoeia (*5*).

J. communis berries are widely used in flavors, perfumes, and pharmaceuticals and to aromatize alchoolic beverages. In particular, they are employed with other botanical ingredients in the production of commonly consumed juniper-based spirits, such as gin (*3, 6*). *J. communis* berries also serve as seasoning and for pickling meat, and roasted and ground berries are utilized as a coffee substitute.

J. communis berries are used in folk medicine for their diuretic, antiseptic, stomachic, and carminative properties (*7*). Moreover, the decoction of branches with berries is drunk to relieve kidney infections (*8*). Berries of *J. communis* var. *saxatilis*

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(*J. communis* ssp. *nana*) are swallowed as pills internally for cough, pain, and treatment of hemorrhoids in Turkish traditional medicine (*9*).

Over the years, the phytochemistry of *Juniper* genus has been largely studied; however, concerning *J. communis* L. berries most of studies focused on the chemical composition of the essential oil (*10, 11*). Recently Innocenti et al. (*12*) for the first time have reported the presence of flavonoids and biflavonoids in *J. communis* L. berries from Italy.

Since *J. communis* var. *communis* and *J. communis* var. *saxatilis* berries are indistinctly utilized in Turkish folk medicine, it seemed interesting to define and compare the flavonoid composition and the biological potential of the berries of these two varieties from Turkey. For these purposes, the flavonoid profile was established by HPLC-DAD−ESI-MS analysis. Moreover, total polyphenols were spectrophotometrically determined. The antioxidant properties of methanol extracts of the two varieties were examined by means of different in vitro systems, with the aim to hypothesize whether they can act as primary and/or secondary antioxidants: the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test, the reducing power assay, and the Ferrous ions (Fe^{2+}) chelating assay. Moreover, the antilipid peroxidation activity of the extracts was assessed by the thiobarbituric acid (TBA) test.

In the recent years, infections have increased to a great extent and resistance against antibiotics becomes an ever-increasing therapeutic problem; thus, natural products of higher plants could give a new source of antimicrobial agents. Since *J. communis* berries are traditionally used for their antiseptic action, it seemed interesting to extend our study to the evaluation of the antimicrobial activity of these two varieties.

MATERIALS AND METHODS

Plant Material. The ripe berries of *J. communis* var. *communis* and *J. communis* L. var. *saxatilis* were collected in Ağrı and Ankara (Turkey), respectively, and identified by Prof. Ayşegül Güvenç at the Department of Pharmaceutical Botany, Ankara University, Turkey. Voucher specimens are deposited at the Herbarium (AEF) of the Faculty of Pharmacy, Ankara University, Turkey, with herbarium numbers AEF 23854 and AEF 23801.

The berries without seeds were dried at room temperature and powdered. The methanol extracts were obtained by subjecting the powdered berries (20 g) at maceration in methanol (200 mL) at 50 °C for 8 h under continuous shaking (700 rpm/min) and in the dark, twice. After filtration, the extractive solutions were evaporated to dryness by rotary evaporator (40 °C). The yields of the extracts of *J. communis* var. *communis* (Jcc) and *J. communis* var. *saxatilis* (Jcs), referenced to 100 g of dried berries, were 22.59% and 25.83%, respectively.

Chemicals. CH_3CN and water, HPLC and HPLC/MS grade, were from VWR International Srl. (Milan, Italy). Formic acid, HPLC/MS grade, was from Riedel-de-Haën (Seelze, Germany). The pure standards of rutin and amentoflavone were supplied from Extrasynthese (Geney, France). $FeCl_2$ was obtained from Carlo Erba (Milan, Italy). Tryptone soya broth, Sabouraud dextrose agar, Müeller Hinton broth, and Müeller Hinton agar were supplied from Oxoid (Basingstoke, UK). Unless indicated otherwise, all chemicals were purchased from Sigma-Aldrich (Milan, Italy).

Determination of Total Polyphenols. The total polyphenol content of Jcc and Jcs extracts was determined by the Folin−Ciocalteu method, referencing to the calibration curve of gallic acid, phenol compound used as a standard (*13*). Briefly, a total of 100 μL of each sample solution was mixed with 0.2 mL pf Folin−Ciocalteu reagent, 2 mL of H_2O, and 1 mL of 15% Na_2CO_3, and the absorbance was measured at 765 nm, after 2 h incubation at room temperature, with a model UV-1601 spectrophotometer (Shimadzu, Milan, Italy). The total polyphenols were estimated as gallic acid equivalent (GAE) and expressed in mg GAE/g extract (dw) ± standard deviation (SD). The data were obtained from the average of three determinations.

Identification of Flavonoid Compounds by HPLC-DAD−ESI-MS Analysis. A qualitative−quantitative investigation of flavonoids contained in Jcc and Jcs extracts has been carried out by HPLC-DAD−ESI-MS analysis. The extracts were dissolved in methanol (10 mg/mL Jcc and 20 mg/mL Jcs) and filtered through 0.45 μm membrane filters (Whatman, Clifton, NJ). The analyses were carried out using a Shimadzu HPLC-DAD−ESI-MS system (Kyoto, Japan), equipped with a SCL-10Avp controller, two LC-10ADvp pumps, and a photodiode detector SPD-M10A vp. In addition, an IT-TOF mass spectrometer (Shimadzu) with an electrospray interface (ESI) was employed. The column used was a Discovery HS C_{18} (15 cm × 2.1 mm i.d., 3 μm) purchased from Supelco (Bellefonte, PA).

The mobile phases were (A) 0.1% formic acid/water and (B) CH_3CN, with the following multistep linear solvent gradient: 0−15 min, 10−20% B; 15−25 min, 20−35% B; 25−35 min, 35−50% B; 35−40 min, 50−100% B; 40−45 min, 100% B. The flow rate was 0.2 mL/min, and the injection volume of the samples was 2 μL.

UV−vis spectra were recorded in the wavelength range of 200−500 nm, and the chromatograms were acquired at 350 nm. Mass spectra were recorded in negative ion mode. The mass spectrometer operating conditions were as follows: nitrogen flow rate, 1.5 L/min; curved desolvation time (CDL) temperature, 300 °C; block temperature, 200 °C; ion accumulation, 100 ms. The spectra were acquired in the m/z range of 200−870 amu.

Flavonoids and biflavonoids were identified through the use of pure commercial standards and by comparison of retention times, UV spectra, and mass spectra with bibliographic data (*12, 14*). The quantitative determination of each compound was carried out using the external standard method. All of the flavonoids were calculated at 350 nm using a standard of rutin in a range of concentrations between 5 and 100 (μg/mL). The biflavonoid amounts were calculated at 350 nm using amentoflavone as reference compound in the range of 5−100 (μg/mL). The results were obtained from the average of three determinations and are expressed in μg/g extract (dw) ± standard deviation (SD).

Antioxidant Activity. *Free Radical Scavenging Activity*. Free radical scavenging activity of Jcc and Jcs extracts has been evaluated using the DPPH test (*15*). An aliquot (0.5 mL) of methanol solution containing different amounts of each extract (0.07−2 mg/mL) was added to 3 mL of daily prepared methanol DPPH solution (0.1 mM). The optical density change at 517 nm was measured, 20 min after the initial mixing, with a model UV-1601 spectrophotometer (Shimadzu). Butylated hydroxytoluene (BHT) was used as reference.

The scavenging activity was measured as the decrease in absorbance of the samples versus DPPH standard solution. Results were expressed as radical scavenging activity percentage (%) of the DPPH, defined by the formula $[(A_o − A_c)/A_o] × 100$, where A_o is the absorbance of the control and A_c is the absorbance in the presence of the sample or standard. The assays were carried out in triplicate, and the results are expressed as mean values ± standard deviation (SD). The results were also expressed as mean inhibiting concentration (IC_{50}); this parameter is defined as the concentration of substrate necessary to scavenge 50% DPPH free radicals, and it was calculated by using the Litchfield and Wilcoxon test (*16*).

Reducing Power Assay. The reducing power of Jcc and Jcs extracts was determined according to the method of Oyaizu (*17*). Different amounts of Jcc and Jcs extracts (0.07−2 mg/mL) in 1 mL of solvent were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferrycyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50 °C for 20 min. The resulting solution was cooled rapidly, mixed with 2.5 mL of 10% trichloroacetic acid, and centrifuged at 3000 rpm for 10 min. The resulting supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% fresh $FeCl_3$, and the absorbance was measured, after 10 min, at 700 nm with a model UV-1601 spectrophotometer (Shimadzu); increased absorbance of the reaction mixture indicated increased reducing power. As blank, an equal volume (1 mL) of water was mixed with a solution prepared as described above. Ascorbic acid and BHT were used as reference.

The assays were carried out in triplicate, and the results are expressed as mean absorbance values ± standard deviation (SD). The reducing power was also expressed as ascorbic acid equivalent (ASE/mL). When the reducing power is 1 ASE/mL, the reducing power of 1 mL extract is equivalent to 1 μmol of ascorbic acid (*18*).

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Ferrous Ions (Fe²⁺) Chelating Activity. The chelating activity of ferrous ions by the Jcc and Jcs extracts was estimated by the method of Dinis et al. (*19*). Briefly, extracts (0.07−2 mg/mL) in 0.4 mL of methanol were added to a solution of 2 mM FeCl₂ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL), and the total volume was adjusted to 4 mL with methanol. The mixture was shaken vigorously and left standing at room temperature for 10 min; absorbance of the solution was then measured spectrophotometrically at 562 nm (UV-1601 spectrophotometer, Shimadzu). The percentage of inhibition of the ferrozine−(Fe²⁺) complex formation was calculated with the formula $[(A_o - A_c)/A_o] \times 100$, where A_o is the absorbance of the control and A_c is the absorbance in the presence of the sample or standard. The control contains FeCl₂ and ferrozine, complex formation molecules. BHT was used as a reference standard. The assays were carried out in triplicate, and the results are expressed as mean values ± standard deviation (SD).

Thiobarbituric Acid (TBA) Test. In order to assess the efficacy of the extracts to protect liposomes from lipid peroxidation, the TBA test was used (*20*). Briefly, extracts were redissolved in methanol and tested at different concentrations (0.016−1 mg/mL). Liposomes were prepared from bovine brain extract in phosphate buffered saline (5 mg/mL). Peroxidation was started by adding 0.1 mL of FeCl₃ (1 mM) and 0.1 mL of ascorbic acid (1 mM), followed by incubation at 37 °C for 20 min. BHT was added to prevent lipid peroxidation during the TBA test itself. The absorbance of the upper layers, which contain the chromogen, was determined spectrophotometrically at 532 nm. Propyl gallate was used as a reference compound. The percentage of lipid peroxidation inhibition was assessed by using the following formula: $[(FRM - B) - (ET - B - EA)/(FRM - B)] \times 100$, where FRM is the absorbance of the control reaction and ET is absorbance in the presence of the sample. The absorbance of liposomes alone (B) and extract alone (EA) was also taken into account.

The assays were carried out in triplicate, and the results are expressed as mean values ± standard deviation (SD). The mean inhibiting concentration (IC₅₀) of the extracts was calculated (*16*).

Antimicrobial Activity. *Microbial Strains and Culture Conditions.* The following strains were used as indicators for antimicrobial testing and were obtained from the in-house culture collection of Pharmaco-Biological Department (University of Messina, Italy): *Staphylococcus aureus* strain ATCC 8538P, *Staphylococcus epidermidis* G1, *Enterococcus hirae* V3, *Bacillus subtilis* P3, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9021, *Proteus mirabilis* G4 were grown at 37 °C in Tryptone soya broth; *Candida albicans* ATCC10231 and *Candida parapsilosis* (P7) were grown at 35 °C on Sabouraud dextrose agar (SDA).

MIC and MBC Determinations. The minimum inhibitory concentrations (MICs) of Jcc and Jcs extracts were determined in Müeller Hinton broth (MHB) using a broth dilution micromethod in polystyrene microtiter plates according to the Clinical Laboratory Standards Institute (CLSI) guidelines (*21*). The microplates were maintained under shaking conditions to prevent sedimentation and to increase aeration. The final concentrations of the extracts adopted to evaluate the antibacterial activity were 2.44−2500 μg/mL. Two growth controls consisting of MHB medium and MHBMeOH were included. Microbial growth was determined by visual readings and spectrophotometric values (550 nm) using the microplate reader, model 550 (BIO-RAD Laboratories Milano, Italy). The MIC was considered as the lowest concentration of each extract at which there was no microbial growth after 24 h of incubation.

As an indicator of bacterial growth, 20 μL of 2,3,5-triphenyl-2*H*-tetrazolium chloride (TTC) dissolved in water was added to the wells and incubated at 37 °C for 30 min (*22*). Where bacterial growth was inhibited, the solution in the well remained clear after incubation with TTC. The minimum bactericidal concentrations (MBCs) were determined by seeding 20 μL from all clear MIC wells onto Müeller Hinton agar (MHA) plates. The MBC was defined as the lowest extract concentration that killed 99.9% of the final inoculums after 24 h of incubation. The data from at least three replicates were evaluated, and modal results were calculated.

MIC and MFC Determinations. The minimum inhibitory concentrations (MICs) were determined in RPMI1640 buffered to a pH of 7.0 with MOPS according to National Committee for Clinical Laboratory Standards (NCCLS M27-A2) (*23*). The microplates were maintained under shaking conditions to prevent sedimentation and to increase
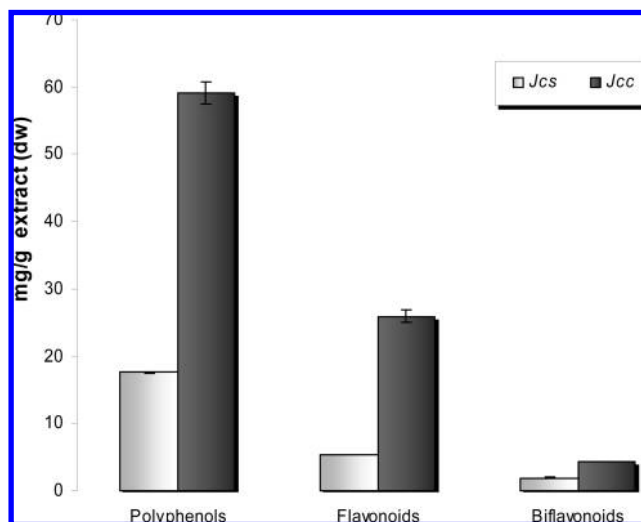

Figure 1. Quantitative determination of total polyphenols (calculated as gallic acid), flavonoids (calculated as rutin), and biflavonoids (calculated as amentoflavone) of *J. communis* var. *communis* (Jcc) and *J. communis* var. *saxatilis* (Jcs) berries methanol extracts. Values are expressed as the mean ± SD (*n* = 3).

aeration. The final concentrations of the extracts adopted to evaluate the antibacterial activity were 2.44−2500 μg/mL. Two growth controls consisting of RPMI 1640 medium and RPMI1640MeOH were included. Microbial growth was determined by visual readings and spectrophotometric values (550 nm) using the microplate reader (BIO-RAD). The MIC was considered as the lowest concentration of each extract at which there was no microbial growth after 24−48 h of incubation.

The minimum fungicidal concentrations (MFCs) were determined by seeding 20 μL from all clear MIC wells onto SDA plates. The MFC was defined as the lowest extract concentration that killed 99.9% of the final inoculums after 24−48 h of incubation. The data from at least three replicates were evaluated, and modal results were calculated.

RESULTS AND DISCUSSION

Determination of Total Polyphenols. The total polyphenol content was 59.17 ± 1.65 mg GAE/g extract in Jcc and 17.64 ± 0.09 mg GAE/g extract in Jcs (**Figure 1**).

Identification of Flavonoid Compounds by HPLC-DAD−ESI-MS Analysis. Figure 2 shows HPLC-DAD chromatograms, extracted at 350 nm, of flavonoid compounds contained in Jcc and Jcs extracts.

The identified flavonoids, mostly in glycosylated form, were *O*-glycoside and *C*-glycoside derivatives of flavones (apigenin, isoscutellarein, hypolaetin, and gossypetin) and of flavonols (quercetin). The sugar moiety was represented by pentoses or hexoses that were not identified. With regard to the class of biflavonoids, the overlapping of the UV−vis spectrum of apigenin with those of the biflavonoid derivatives confirmed the presence of biapigenin compounds. Peaks were identified by comparison with literature data and, with regard to amentoflavone, through the use of pure commercial standard (*12, 14*).

Among biflavonoids, cupressoflavone, amentoflavone, hynokiflavone, and other biflavones and methylbiflavones were detected. Furthermore, the use of the IT-TOF detector allowed confirmation of the identification of the flavonoid compounds in the two samples. In particular, most of these compounds presented a good correlation between theoretical and experimental molecular mass results. The higher sensitivity of the hybrid mass spectrometer allowed identification of some flavonoids after the loss of the sugar moiety as well as biflavonoids with accuracy values lower than 4.87 ppm, as can be seen in **Table 1**. The exact

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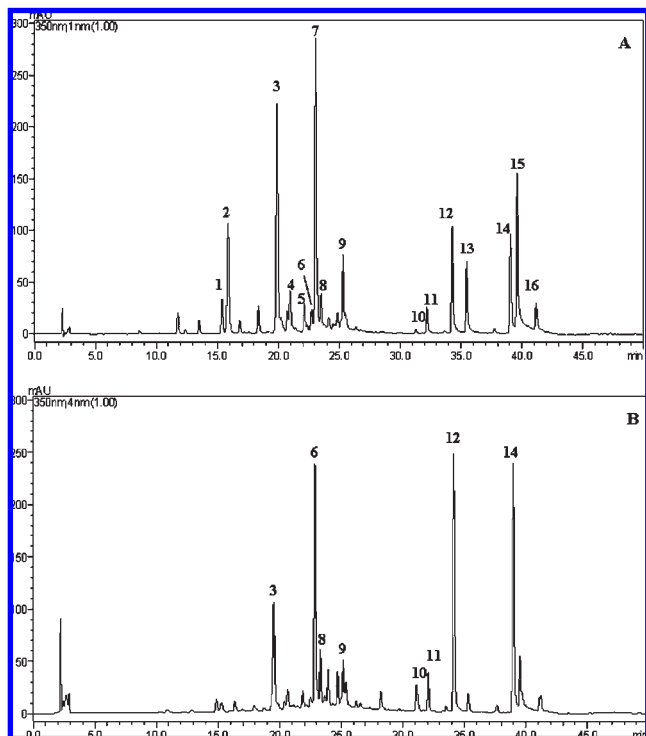

Figure 2. HPLC-DAD chromatograms (extracted at 350 nm) of the flavonoids of *J. communis* var. *communis* (Jcc) (**A**) and *J. communis* var. *saxatilis* (Jcs) (**B**) berries methanol extracts. The extracts of Jcc and Jcs were analyzed at concentrations of 10 and 20 mg/mL, respectively. For peak identification, see **Table 1**.

mass identification proposed in this contribution has never been reported for *J. communis* L. berries.

The HPLC analysis of Jcc and Jcs extracts has shown quantitative differences in the flavonoid content. In fact, Jcc exhibited a higher concentration of total flavonoids (25947 ± 0.86 μg/g extract) and biflavonoids (4346 ± 3.95 μg/g extract) compared to Jcs (5387 ± 34.88 and 1944 ± 26.88 μg/g extract, respectively) (**Figure 1**). The results obtained by HPLC analysis are in agreement with the data obtained by the Folin−Ciocalteu method.

The results of HPLC analysis also showed qualitative differences in the flavonoid profile of the extracts. The analysis of Jcc extract allowed the separation of 16 different compounds, 10 flavonoids, and 6 biflavonoids. The identified flavonoid compounds were hexosides and pentosides of quercetin, isoscutellarein, hypolaetin, apigenin, and gossypetin. Among flavonoids, hypolaetin-7-pentoside (7986 ± 7.73 μg/g extract) and quercetin-hexoside (7381 ± 1.75 μg/g extract) are present in greater amounts, whereas amentoflavone (980 ± 7.50 μg/g extract) and a methylbiflavone (1170 ± 5.68 μg/g extract) represent the most abundant biflavonoid compounds (**Table 2**). Most of the compounds identified in Jcc berries from Turkey are present in Italian *J communis* berries, too; nonetheless, some differences in the flavonoid profile of Turkish Jcc berries were highlighted. In fact, with respect to ref *12*, three different flavonoid compounds have been detected. On the basis of UV and MS spectra as well as retention behavior, two out of three were tentatively identified as gossypetin-hexoside-pentoside (peak 1) and gossypetin-hexoside (peak 2). To the best of our knowledge, there are no published reports for the presence of these flavonoids in *J. communis* berries.

Jcs appeared less rich in flavonoids, both qualitatively and quantitatively; in fact HPLC analysis of Jcs extract led to the

Table 1. List of Flavonoids Identified in *J. communis* var. *communis* (Jcc) and *J. communis* var. *saxatilis* (Jcs) Berries Methanol Extracts

peak	compd	λ_{max} (nm)	formula [M]/[aglycone]	[M − H]⁻/ [aglycone − H]⁻ calcd (monoisotopic)	[M − H]⁻/ [aglycone − H]⁻ obsd, Jcc	accuracy (ppm)	[M −H]⁻/ [aglycone − H]⁻ obsd, Jcs	accuracy (ppm)	refs
1	gossypetin-hexoside-pentoside	273, 370	$C_{26}H_{27}O_{17}$	611.124830	611.1223	4.13			*14*
			$C_{21}H_{19}O_{13}$	479.082570	479.08114	2.98			
			$C_{20}H_{15}O_{12}$	447.056355	447.05464	3.84			
			$C_{15}H_9O_8$	317.029745	317.02845	4.08			
2	gossypetin-hexoside	271, 372	$C_{21}H_{19}O_{13}$	479.082570	479.0810	3.27			*14*
			$C_{15}H_9O_8$	317.029745	317.0287	3.29			
3	quercetin-hexoside	255, 353	$C_{21}H_{20}O_{12}$	463.087655	463.0854	4.87	463.0874	0.55	*12*
			$C_{15}H_{10}O_7$	301.034829	301.0336	4.08	301.0343	1.76	
4	quercetin-hexoside	255, 353	$C_{21}H_{20}O_{12}$	463.087655	463.0862	3.14			*14*
			$C_{15}H_{10}O_7$	301.034829	301.0336	4.08			
5	quercetin-3-*O*-pentoside	276, 343	$C_{20}H_{18}O_{11}$	433.07709	433.0756	4.87			*12*
			$C_{15}H_{10}O_7$	301.034829	301.0337	3.75			
6	isoscutellarein-8-*O*-hexoside	276, 305, 329	$C_{21}H_{20}O_{11}$	447.092739	447.0921	1.43	447.0926	0.31	*12*
			$C_{15}H_{10}O_6$	285.039915			285.0400	0.30	
7	hypolaetin-7-pentoside	202, 267, 344	$C_{20}H_{18}O_{11}$	433.07709	433.0756	3.44			*12*
			$C_{15}H_{10}O_7$	301.034829	301.0336	4.08			
8	apigenin-hexoside	233, 267, 342	$C_{21}H_{20}O_{10}$	431.097824	431.0964	3.30	431.0965	3.07	*12*
9	isoscutellarein-7-*O*-pentoside	277, 306, 327	$C_{20}H_{18}O_{10}$	417.082175	417.0837	3.65	417.0818	0.90	*12*
			$C_{15}H_{10}O_6$	285.039915	285.0406	2.40	285.0406	2.40	
10	apigenin	235, 272, 342	$C_{15}H_{10}O_5$	269.045	269.0460	3.71	269.0456	2.23	*12*
11	cupressoflavone	232, 273, 334	$C_{30}H_{18}O_{10}$	537.082175	537.0807	2.70	537.0831	1.72	*12*
12	amentoflavone	230, 270, 337	$C_{30}H_{18}O_{10}$	537.082175	537.0813	1.63	537.0842	3.77	*12*
13	biflavone	234, 270, 342	$C_{30}H_{18}O_{10}$	537.082175	537.0811	2.00			*12*
14	methylbiflavone	232, 270, 334	$C_{31}H_{20}O_{10}$	551.097825	551.0963	2.77	551.0962	2.95	*12*
15	methylbiflavone	234, 271, 338	$C_{31}H_{20}O_{10}$	551.097825	551.0967	2.04			*12*
16	Hhynokiflavone	240, 286, 336	$C_{30}H_{18}O_{10}$	537.082175	537.0817	0.88			*12*

Table 2. Quantitative Determination of Flavonoids (Calculated as Rutin) and Biflavonoids (Calculated as Amentoflavone) of *J. communis* var. *communis* (Jcc) and *J. communis* var. *saxatilis* (Jcs) Berries Methanol Extracts[a]

		μg/g extract (mean ± SD)	
peak	compd	Jcc	Jcs
1	gossypetin-hexoside-pentoside	925 ± 1.48	
2	gossypetin-hexoside	3172 ± 13.76	
3	quercetin-hexoside	7381 ± 1.75	1602 ± 11.02
4	quercetin-hexoside	946 ± 0.82	
5	quercetin-3-*O*-pentoside	1013 ± 10.81	
6	isoscutellarein-8-*O*-hexoside	631 ± 1.43	2435 ± 7.51
7	hypolaetin-7-pentoside	7986 ± 7.73	
8	apigenin-hexoside	1275 ± 0.79	549 ± 4.27
9	isoscutellarein-7-*O*-pentoside	2591 ± 5.11	535 ± 3.52
10	apigenin	26 ± 0.75	266 ± 8.56
11	cupressoflavone	334 ± 0.26	146 ± 7.92
12	amentoflavone	980 ± 7.50	946 ± 17.32
13	biflavone	708 ± 6.24	
14	methylbiflavone	811 ± 1.27	852 ± 1.65
15	methylbiflavone	1170 ± 5.68	
16	hynokiflavone	343 ± 0.54	

[a] Values are expressed as the mean ± SD (*n* = 3).

identification of eight compounds, including five flavonoids and three biflavonoids. The most abundant components are isoscutellarein-8-*O*-hexoside (2435 ± 7.51 μg/g extract) and quercetin-hexoside (1602 ± 11.02 μg/g extract) among flavonoids, amentoflavone (946 ± 17.32 μg/g extract) and a methylbiflavone (852 ± 1.65 μg/g extract) among biflavonoids (**Table 2**).

It is well-known that the final composition of flavonoids and other phenolic compounds in fruits and other parts of the plants is determined both genetically and by environmental factors (botanical origin, water, nutrient, and light conditions). It has been previously reported that *J. communis* var. *communis* and *J. communis* var. *saxatilis* are strongly genetically related and that the monoterpene pattern is related to geographical origin and not to the species identity (*4*). Hence, the environmental factors surely have also a strong influence on the flavonoid pattern of the two varieties.

Comparing the flavonoid composition of the two studied varieties of *J. communis* reveals the presence of gossypetin-hexoside-pentoside, gossypetin-hexoside, quercetin-3-*O*-pentoside, hypolaetin-7-pentoside, and hynokiflavone only in the Jcc extract; hence, the obtained results imply further investigation directed toward testing the possibility of chemosystematic distinguishing between the two varieties based on the presence of these compounds in Jcc berries.

Antioxidant Activity. It is known that the antioxidant activity can be primary and secondary: primary antioxidants scavenge radicals to inhibit chain initiation and break chain propagation; secondary antioxidants suppress the formation of radicals and protect against oxidative damage (*24*). In this study we evaluated primary antioxidant properties by DPPH test and reducing power assay and we evaluated the secondary antioxidant ability by ferrous ions (Fe^{2+}) chelating activity. Moreover, TBA assay was performed to measure liposome antilipid peroxidation.

Oxidative stress occurs when free radical formation exceeds the body's ability to protect itself. Reactive species such as hydroxyl radicals and nitric oxide radicals have been widely implicated in the etiology of several diseases. Removal of these reactive oxygen species or suppression of their generation is an efficient way of controlling these diseases (*24*).

DPPH test has been widely used to evaluate the free radical scavenging effects of natural antioxidants. The radical scavenging activity of an extract is linked to the ability to donate a proton.
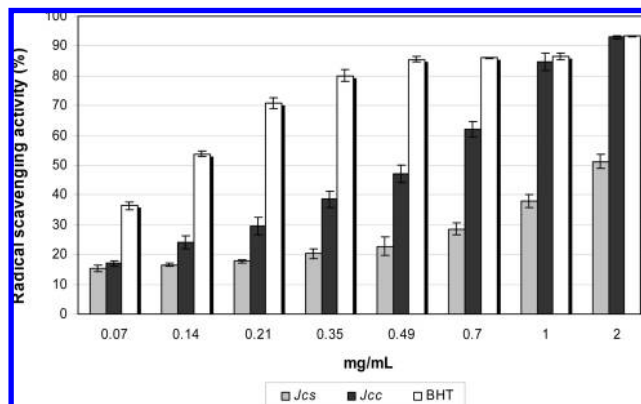

Figure 3. Free radical scavenging activity of *J. communis* var. *communis* (Jcc) and *J. communis* var. *saxatilis* (Jcs) berries methanol extracts. Each value is expressed as the mean ± SD (*n* = 3).

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable radical in methanol with violet color because of delocalization of the spare electron throughout the molecule. When a proton is accepted in the reaction with the oxygen atom of a radical scavenger's OH group, the reduced DPPH-H (2,2-diphenyl-1-picrylhydrazine) is formed, which is yellow (*25*). The degree of discoloration indicates the amount of DPPH scavenged; the greater is the bleaching action, the higher is the antioxidant activity, and this is reflected in a lower IC_{50} value.

Jcc and Jcs extracts showed free radical scavenging activity, which was much higher in Jcc than in Jcs (**Figure 3**). Furthermore, a dose−response relationship in the DPPH radical scavenging activity of both extracts is evident; in fact, the activity increases with increasing concentrations. The IC_{50} values of the extracts (0.63 ± 0.09 mg/mL and 1.84 ± 0.10 mg/mL, respectively) are higher with respect to BHT (IC_{50} = 0.12 ± 0.01 mg/mL); anyway, Jcc extract, at the concentrations of 1 and 2 mg/mL, showed an activity comparable to that of BHT.

The reducing power reflects the ability to stop the radical chain reaction and can be considered a significant indication of potential antioxidant activity of an extract. In the assay for the determination of reducing power the presence of antioxidants compounds in the sample determines the reduction of Fe^{3+} to the ferrous form; this reduction is highlighted by spectrophotometric measurement of the intensity of Perl's Prussian blue obtained, which depends on the reducing ability of the extract. The higher is the absorbance, the greater is the reducing power, and this is reflected in a lower ASE/mL value (*25*). **Figure 4** reports the absorbance values of Jcc and Jcs; both extracts showed a moderate reducing power, and Jcc was more active than Jcs extract, as also indicated by the ASE values (12.82 ± 0.10 ASE/mL and 64.14 ± 1.20 ASE/mL, respectively). Like the radical scavenging activity, the reducing power of the extracts was dose dependent. Both extracts showed a lower reducing power than BHT (1.75 ± 0.05 ASE/mL).

Iron is an essential mineral for normal physiology, but an excess of it may result in cellular injury. If it undergoes the Fenton reaction, this reduced metal may form highly reactive hydroxyl radicals and thereby contribute to oxidative stress. The resulting oxy radicals cause damage to cellular lipids, nucleic acids, proteins, carbohydrates and lead to cellular impairment. It was reported that chelating agents, which form σ-bonds with a metal, are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of metal ion. Since ferrous ions are the most effective pro-oxidants in the food system, the good chelating effect would be beneficial and removal of free iron from
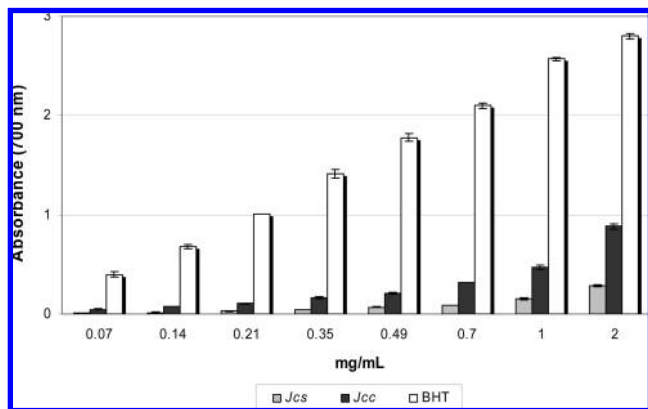
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Figure 4. Reducing power of *J. communis* var. *communis* (Jcc) and *J. communis* var. *saxatilis* (Jcs) berries methanol extracts (spectrophotometric detection of $Fe^{3+}-Fe^{2+}$ transformation). Each value is expressed as the mean $\pm$ SD ($n = 3$).
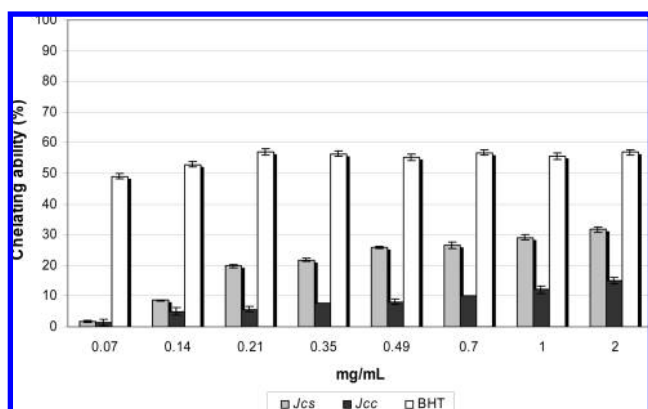

Figure 5. Ferrous ions (Fe^{2+}) chelating activity of *J. communis* var. *communis* (Jcc) and *J. communis* var. *saxatilis* (Jcs) berries methanol extracts. Each value is expressed as the mean $\pm$ SD ($n = 3$).

circulation could be a promising approach to prevent oxidative stress-induced diseases (25, 26).

The method of metal chelating activity is based on chelating of Fe^{2+} ions by the reagent ferrozine, which can quantitatively form complexes with Fe^{2+}; in the presence of chelating agents, the complex formation is disrupted, with the result that the red color of the complex is decreased. Measurement of color reduction allows estimation of the chelating activity of the coexisting chelator (26). In this assay Jcc and Jcs extracts interfered with the formation of the ferrous complex with the reagent ferrozine, suggesting that they possess chelating activity. Both extracts showed a moderate Fe^{2+} chelating ability, which was more elevated in Jcs than in Jcc; moreover, the chelating activity of the extracts is dose-dependent. Finally, the extracts exerted lower chelating effects on ferrous ions than those of the standard BHT (**Figure 5**).

The TBA reaction is based on the fact that peroxidation of most membrane systems leads to formation of small amounts of free malonaldehyde (MDA). One molecule of MDA reacts with two molecules of TBA to yield a colored product, which in an acid environment absorbs light at 532 nm and is readily extractable into organic solvents. The intensity of color is proportional to the concentration of MDA; higher MDA levels indicate an increased lipid peroxidation. The incorporation of any antioxidant compound in the lipid peroxidation assay reaction mixture will lead to a reduction of the extent of peroxidation (20). In the TBA

method, Jcc was more active than Jcs extract (IC$_{50}$ of 4.44 $\pm$ 0.70 μg/mL and 120.07 $\pm$ 3.60 μg/mL). However, both extracts showed lower antioxidant activity than propyl gallate (IC$_{50}$ of 0.21 $\pm$ 0.40 μg/mL).

On the basis of the results obtained, it is evident that Jcc and Jcs extracts can act as both primary and secondary antioxidants. The primary antioxidant activity observed seems to be related to the total polyphenol content of the extracts, thus demonstrating that polyphenols, free or glycosilated, play an important role in the protection against free radicals. Phenol compounds are an important group of active principles that act as primary antioxidants; these compounds, as is known, may react with oxygen radicals, as hydroxyl radicals, superoxide anions, and peroxyl radical, and inhibit the lipid oxidation at an early stage (27).

The radical scavenging activity of flavonoids has been thoroughly demonstrated. Jcc extract contains high amounts of the flavonoids quercetin-hexoside and hypolaetin-7-pentoside and of the biflavonoid amentoflavone; several bibliographic data report the radical scavenging properties of aglicons quercetin and hypolaetin and their glycosides and of amentoflavone (28, 29). Therefore, the primary antioxidant activity of the extracts could be related, almost in part, to the presence of these flavonoid compounds.

Anyway, *J. communis* berries also contain several compounds belonging to the category of catechins (flavan-3-ols) as (+)-afzelechin, (−)-epiafzelechin, (+)-catechin, (−)-epicatechin, (+)-gallocatechin, and (+)-epigallocatechin (30). Hence, these polyphenol compounds contained in the extracts could be involved in the observed effects, too.

With regard to secondary antioxidant properties, the results of metal chelating activity are in contrast with those of the other antioxidant tests, where Jcc extract was more active. As previously reported, the total polyphenol content of Jcs extract was lower than that of Jcc; so it could be possible to provide an explanation for the more elevated secondary antioxidant properties of Jcs with respect to Jcc by evaluating its total phytochemical profile. Thus, the chelating ability may be ascribed to other phytochemicals that are extracted together with polyphenols.

The results of the determination of lipid peroxidation showed that Jcc was much more active than Jcs extract; these data were in accordance with those of primary antioxidant activity tests. The antilipid peroxidation effects observed could be correlated to the different polyphenol content of the extracts and mainly to flavonoids. In lipid oxidation models, peroxyl-radical scavenging and transition metal (usually iron) chelation properties are very important mechanistic factors, and it is well-known that flavonoids possess both excellent iron-chelating and radical scavenging properties. However, the strong difference in the activity of the extracts can be only partly related to the polyphenol content. Because of the chemical complexity of extracts, probably other phytochemicals are involved in the observed effects. In addition, other phenomena, as synergism, could be involved in the antioxidant response. In this assay, antioxidant compounds could act sinergically and interfere not only with the propagation reactions of the free radical but also with the formation of the radicals by chelating the transition metal involved in the initiation reaction (31).

Antimicrobial Activity. The MIC values of Jcc and Jcs berries extracts against all the bacteria tested and the yeasts are presented in **Table 3**. The results of negative controls containing methanol (maximum 1% v/v) indicate the complete absence of inhibition of all the strains tested (data not shown).

Both methanol extracts showed activity versus the Gram-positive bacteria but were not active against any of the Gram-negative bacteria or the yeasts tested in the study. Among the two

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Table 3. MIC Values of *J. communis* var. *communis* (Jcc) and *J. communis* var. *saxatilis* (Jcs) Berries Methanol Extracts[a]

microorganism	MIC (µg/mL)		
	Jcc	Jcs	standard drug
Gram-positive			
Staphylococcus aureus ATCC 6538P	156.25	156.25	0.3[b]
Staphylococcus epidermidis G1	1250.00	1250.00	0.3[b]
Enterococcus hirae V3	625.00	156.25	0.2[b]
Bacillus subtilis P3	312.50	156.25	0.2[b]
Gram-negative			
Escherichia coli ATCC 25922	−	−	2[c]
Pseudomonas aeruginosa ATCC 9021	−	−	8[c]
Proteus mirabilis G4	−	−	2[c]
yeast			
Candida albicans ATCC10231	−	−	0.25[d]
Candida parapsilosis P7	−	−	1.15[d]

[a] The dash (−) indicates not active. [b] Standard drug: ofloxacin. [c] Standard drug: tetracycline. [d] Standard drug: amphotericin B.

extracts, Jcs was found to be the most effective. *S. aureus*, *E. hirae*, *B. subtilis* were the most sensitive strain (complete inhibition achieved with a concentration of 156.25 µg/mL). Jcc showed varying degrees of activity against the strains tested. The most sensitive strain was *S. aureus* (156.25 µg/mL), followed by *B. subtilis* (312.50 µg/mL) and *E. hirae* (625.00 µg/mL). Both extracts were less active versus *S. epidermidis* (1250.00 µg/mL). The inhibitory effect of both extracts against all the strains tested was bacteriostatic rather than bactericidal (data not shown).

Polyphenols, particularly flavonoids, have been found to be effective antimicrobial agents against a wide array of microorganisms (*32*). Investigations into the mechanism of action of flavonoids have shown that these compounds have multiple cellular targets rather than one specific site of action (*32*). The activity of apigenin, for example, has been at least partially attributed to inhibition of DNA gyrase (*33*). The higher antimicrobial activity of Jcs with respect to Jcc could be partly due to the greater amount of apigenin, which was 10-fold more elevated in Jcs with respect to Jcc. Certainly the antimicrobial activity of investigated extracts depend not only on phenolic compounds but also on other different secondary metabolites such as terpenoids, whose antimicrobial activity has been widely confirmed (*4, 9*).

Conclusion. This is the first report on the comparative analysis of the flavonoid composition, the antioxidant and antimicrobial properties of the berries of *J. communis* var. *communis* and *J. communis* var. *saxatilis* from Turkey. The presence of gossypetin-hexoside-pentoside and gossypetin-hexoside in *J. communis* var. *communis* berries is reported for the first time.

On the basis of the results of this investigation, *J. communis* var. *communis* and *J. communis* var. *saxatilis* berries from Turkey could represent potential sources of natural antioxidants and of antimicrobial agents. The highlighted differences in the activity of the extracts are not only dependent on the different polyphenol content; therefore, a bioassay-guided fractionation procedure of the extracts is needed to characterize and isolate the active constituents, aimed at more specific employment of these two varieties from Turkey.

LITERATURE CITED

(1) Franco, J. A.; Juniperus, L. In *Flora Europea*; Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M., Webb, D. A., Eds.; Cambridge University Press: Cambridge, U.K., 1968; Vol. *I*, p 38.

(2) Thomas, P. A.; El-Barghathi, M.; Polwart, A. Biological flora of the British Isles: *Juniperus communis* L. *J. Ecol.* **2007**, *95*, 1404–1440.

(3) Farjon, A.; Juniperus, L. In *Flora of Turkey and the East Aegean Islands*; Güner, A., Özhatai, N., Ekim, T., Başer, K. H. C., Eds.; Edinburgh University Press: Edinburgh, U.K., 2000; Vol. *11*, Suppl. 2, pp 8–10.

(4) Filipowicz, N.; Piotrowski, A.; Ochocka, J. R.; Asztemborska, M. The phytochemical and genetic survey of common and dwarf Juniper (*Juniperus communis* and *Juniperus nana*) identifies chemical races and close taxonomic identity of the species. *Planta Med.* **2006**, *72*, 850–853.

(5) *European Pharmacopoeia*, 6th ed.; European Directorate for the Quality of Medicines & Health Care, Council of Europe: Strasbourg, France, 2008; Vol. *3*, p 2206.

(6) Vichi, S.; Riu-Aumatell, M.; Mora-Pons, M.; Guadayol, J. M.; Buxaderas, S.; Lopez-Tamames, E. HS-SPME coupled to GC/MS for quality control of *Juniperus communis* L. berries used for gin aromatization. *Food Chem.* **2007**, *105* (4), 1748–1754.

(7) Stanić, G.; Samaržija, I.; Blažević, N. Time-dependent diuretic response in rats treated with Juniper berry preparations. *Phytother. Res.* **1998**, *12*, 494–497.

(8) Ritch-Krc, E. M.; Thomas, S.; Turner, N. J.; Towers, G. H. N. Carrier herbal medicine: traditional and contemporary plant use. *J. Ethnopharmacol.* **1996**, *52*, 85–94.

(9) Fujita, T.; Sezik, E.; Tabata, M.; Yeşilada, E.; Honda, G.; Takeda, Y.; Tanaka, T.; Takaishi, Y. Traditional medicine in Turkey VII. Folk medicine in middle and west black sea regions. *Econ. Bot.* **1995**, *49*, 406–422.

(10) Barjaktarović, B.; Sovilj, M.; Knez, Ž. Chemical composition of *Juniperus communis* L. fruits supercritical CO_2 extracts: Dependence on pressure and extraction time. *J. Agric. Food Chem.* **2005**, *53*, 2630–2636.

(11) Gonny, M.; Cavaleiro, C.; Salgueiro, L.; Casanova, J. Analysis of *Juniperus communis* subsp. *alpina* needle, berry, wood and root oils by combination of GC, GC/MS and ¹³C-NMR. *Flavour Fragrance J.* **2006**, *21*, 99–106.

(12) Innocenti, M.; Michelozzi, M.; Giaccherini, C.; Ieri, F.; Vincieri, F. F.; Mulinacci, N. Flavonoids and biflavonoids in Tuscan berries of *Juniperus communis* L.: detection and quantitation by HPLC-DAD−ESI-MS. *J. Agric. Food Chem.* **2007**, *55* (16), 6596–6602.

(13) Gao, X.; Ohlander, M.; Jeppsson, N.; Bjork, L.; Trajkovski, V. Changes in antioxidant effects and their relationship to phytonutrients in fruit of the sea buckthorn (*Hippophae rhamnoides* L.) during maturation. *J. Agric. Food Chem.* **2000**, *48* (5), 1485–1490.

(14) Petsalo, A.; Jalosen, J.; Tolonen, A. Identification of flavonoids of *Rhodiola rosea* by liquid chromatography−tandem mass spectrometry. *J. Chromatogr., A* **2006**, *1112*, 224–231.

(15) Ohinishi, M.; Morishita, H.; Iwahashi, H.; Shitzuo, T.; Yoshiaki, S.; Kimura, M.; Kido, R. Inhibitory effects of chlorogenic acid on linoleic acid, perossidation and haemolysis. *Phytochemistry* **1994**, *36*, 579–583.

(16) Litchfield, J. T.; Wilcoxon, F. A. A simplified method of evaluating dose-effect. experiments. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99–113.

(17) Oyaizu, M. Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn. J. Nutr.* **1986**, *44*, 307–315.

(18) Apáti, P.; Szentmihályi, K.; Kristó, S. T.; Papp, I.; Vinkler, P.; Szoke, E.; Kéry, A. Herbal remedies of Solidago: correlation of phytochemical characteristics and antioxidative properties. *J. Pharm. Biomed. Anal.* **2003**, *32* (4−5), 1045–1053.

(19) Dinis, T. C. P.; Madeira, V. M. C.; Almeida, L. M. Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors membrane lipid peroxidation and peroxyl radical scavengers. *Arch. Biochem. Biophys.* **1994**, *315*, 161–169.

(20) Conforti, F.; Statti, G. A.; Tundis, R.; Menichini, F.; Houghton, P. J. Antioxidant activity of methanolic extract of *Hypericum triquetrifolium* Turra aerial part. *Fitoterapia* **2002**, *73*, 479–483.

(21) Clinical Laboratory Standards Institute, CLSI, **2000**.

(22) Buwa, L. V.; Staden, J. V. Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. *J. Ethnopharmacol.* **2006**, *103* (1), 139–142.

Article

J. Agric. Food Chem., Vol. 57, No. 15, 2009   **6577**

(23) National Committee for Clinical Laboratory Standards. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*; Approved Standard M27-A2; National Committee for Clinical Laboratory Standards: Wayne, PA, 2002.

(24) Lim, Y. Y.; Lim, T. T.; Tee, J. J. Antioxidant properties of several tropical fruits: A comparative study. *Food Chem.* **2007**, *103* (3), 1003–1008.

(25) Gülçin, I.; Elmastaş, M.; Aboul-Enein, H. Y. Determination of antioxidant and radical scavenging activity of basil (*Ocimum basilicum* L. family Lamiaceae) assayed by different methodologies. *Phytother. Res.* **2007**, *21* (4), 354–361.

(26) Ningappa, M. B.; Dinesha, R.; Srinivas, L. Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf (*Murraya koenigii* L.) extracts. *Food Chem.* **2008**, *106* (2), 720–728.

(27) Chang, L. W.; Yen, W. J.; Huang, S. C.; Duh, P. D. Antioxidant activities of sesame coat. *Food Chem.* **2002**, *78*, 347–354.

(28) Ibrahim, N. A.; El-Seedi, H. R.; Mohammed, M. M. Phytochemical investigation and hepatoprotective activity of *Cupressus sempervirens* L. leaves growing in Egypt. *Nat. Prod. Res.* **2007**, *21* (10), 857–866.

(29) Mariani, C.; Braca, A.; Vitalini, S.; De Tommasi, N.; Visioli, F.; Fico, G. Flavonoid characterization and in vitro antioxidant activity of *Aconitum anthora* L. (Ranunculaceae). *Phytochemistry* **2008**, *69* (5), 1220–1226.

(30) Wren R. C. *Potter's New Cyclopaedia of Botanical Drugs and Preparations*; The C.W. Daniel Company Ltd.: Saffron Walden, U.K., 1987; pp 160−161.

(31) Jayabalan, R.; Subathradevi, P.; Marimuthu, S.; Sathishkumar, M.; Swaminathan, K. Changes in free-radical scavenging ability of kombucha tea during fermentation. *Food Chem.* **2008**, *109*, 227–234.

(32) Cushnie, T. P. T.; Lamb, A. J. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents* **2005**, *26*, 343–356.

(33) Ohemeng, K. A.; Schwender, C. F.; Fu, K. P.; Barrett, J. F. DNA gyrase inhibitory and antibacterial activity of some flavones (1). *Bioorg. Med. Chem. Lett.* **1993**, *3*, 225–230.