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Tea and herbal infusions: Their antioxidant activity and phenolic profile

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Abstract

Tea and herbal infusions have been studied for their polyphenolic content, antioxidant activity and phenolic profile. The total phenolics recovered by ethyl acetate from the water extract, were determined by the Folin–Ciocalteu procedure and ranged from 88.1 ± 0.42 (Greek mountain tea) to 1216 ± 32.0 mg (Chinese green tea) GAE (Gallic acid equivalents)/cup. The antioxidant activity was evaluated by two methods, DPPH and chemiluminescence assays, using Trolox and quercetin as standards. The EC_{50} of herbal extracts ranged from 0.151 ± 0.002 mg extract/mg DPPH (0.38 quercetin equivalents and 0.57 Trolox equivalents), for Chinese green tea, to 0.77 ± 0.012 mg extract/mg DPPH (0.08 quercetin equivalents and 0.13 Trolox equivalents), for Greek mountain tea. Chemiluminescence assay results showed that the IC_{50} ranged from $0.17 \pm 3.4 \times 10^{-3}$ μg extract/ml of the final solution in the measuring cell (1.89 quercetin and 5.89 Trolox equivalents) for Chinese green tea, to $1.10 \pm 1.86 \times 10^{-2}$ g extract/ml of the final solution in the measuring cell (0.29 quercetin and 0.90 Trolox equivalents) for Greek mountain tea. The phenolic profile in the herbal infusions was investigated by LC-DAD-MS in the positive electrospray ionization (ESI^+) mode. About 60 different flavonoids, phenolic acids and their derivatives have been identified.

Keywords: Antioxidant activity; Flavonoids; Phenolic acids; LC–MS; Herbal infusion; Tea

1. Introduction

An antioxidant can be defined as any substance that when present at low concentrations compared to that of an oxidizable substrate, significantly delays or inhibits the oxidation of that substrate (Percival, 1998; Young & Woodside, 2001). The physiological role of free radical- and hydroxyl free radical-scavengers, as this definition suggests, is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals. In recent years, a substantial body of evidence has indicated a key role for free radicals as major contributors to aging and to degenerative diseases of aging, such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction (Ames, Shigenaga, & Hagen, 1990; Percival, 1998; Young & Woodside, 2001). Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants (Percival, 1998). When the availability of antioxidants is limited, this damage can become cumulative and debilitating oxidative stress results (Swanson, 1998). Antioxidants are capable of stabilizing, or deactivating free radicals before the latter attack cells and biological targets. They are therefore critical for maintaining optimal cellular and systemic health and well-being (Percival, 1998) (see Figs. 1 and 2).

Many research groups are examining the chemical nature and activity of natural antioxidants in fruits, vegetables, grains, herbs and other foods (Larson, 1988; Shahidi, 2000). Most antioxidants isolated from higher plants are polyphenols, which show biological activity as

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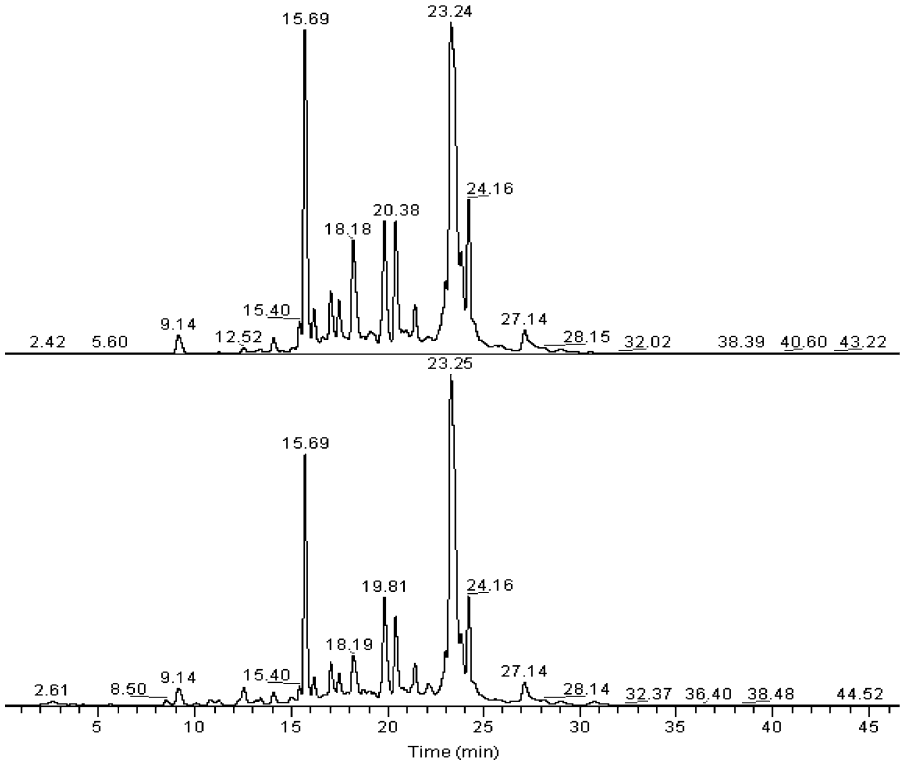

Fig. 1. HPLC chromatogram of Greek mountain tea infusion at 290 and 340 nm, respectively.

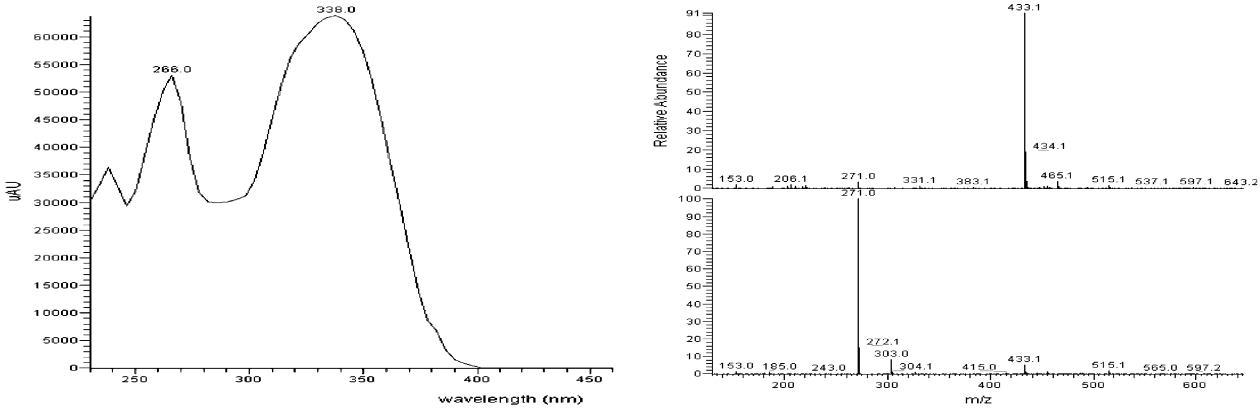

Fig. 2. UV–Vis and ESI-MS (at 20 and 70 eV) spectra of Apigenin 7-xyloside, respectively.

antibacterial, anti-carcinogenic, anti-inflammatory, anti-viral, anti-allergic, estrogenic, and immune-stimulating effects (Larson, 1988). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they have a metal chelation potential. The antioxidant effect of plant phenolics has been studied in relation to the prevention of coronary diseases and cancer, as well as age-related degenerative brain disorders (Parr & Bolwell, 2000).

Tea and herbal infusions contribute to the major source of phenolic compounds in our diet (Shahidi, 2000). Several studies have been conducted for the presence and the activity of antioxidants in tea and herbs but emphasis has been given to organic solvent extracts isolated from dried leaves. Little is known about the phenolic profiles and antioxidant activity in infusions of herbs (Triantaphyllou, Blekas, & Boskou, 2001). The objective of this work was to estimate the phenolic content, evaluate the antioxidant activity and determine the phenolic profile of the water extracts of black and green teas, Greek mountain tea, eucalyptus, linden, sage, chamomile, mint, and dictamnus, which are popular beverages in the Mediterranean region.

2. Materials and methods

2.1. Plant material

The nine different commercial, pre-packaged, dry herbs were purchased from a supermarket in Chania, Crete, Greece.

3. Chemicals and standards

DPPH (2,2-diphenyl-1-picryhydrazyl radicals), EDTA, luminol (3-aminophthalhydrazide), boric acid and Trolox were purchased from Sigma Chemical Co. (Germany). Cobalt (II)[$CoCl_2 \cdot 6H_2O$], Folin–Ciocalteu's reagent, sodium carbonate, ethyl acetate, acetic acid and perhydrol stabilized 30% H_2O_2 were from E Merck (Germany). The methanol used was from Readel de Haën (Germany). Gallic acid and quercetin were from Sigma (USA).

3.1. Preparation of the herbal infusion

Fifteen grams of each herb were infused into 1200 ml of boiling water (equivalent to five teacups) for 3 min, filtered through Whatman No. 4 paper and then concentrated under vacuum to a final volume of 50 ml. Polyphenols from the concentrated samples were extracted twice using ethyl acetate (100 ml × 2). The combined extracts were dried over sodium sulphate, concentrated under vacuum to dryness and the residue obtained was redissolved in 5 ml of methanol for further analyses.

3.2. Determination of total phenolic compounds in the extracts

The amount of total phenolics (TPH) was determined using the Folin–Ciocalteu method (Zheng & Wang, 2001). A calibration curve of gallic acid was prepared, and the results were expressed as mg GAE (gallic acid equivalents)/cup. In this method 5 ml of distilled water were added into a 10 ml volumetric flask. A suitable volume of the herbal extract was transferred into the volumetric flask to obtain absorbance in the range of the prepared calibration curve. About 0.2 ml of Folin–Ciocalteu reagent was added and mixed well. After 3 min, 0.4 ml saturated Na_2CO_3 solution was added, mixed well and made up to volume with distilled water. After a 1 h reaction in the dark, the absorbance was measured at 725 nm using a Hewlett–Packard 8452A diode-array spectrophotometer.

3.3. Evaluation of antioxidant activity of the extracts

In the present study, the antioxidant activity was evaluated in terms of hydrogen donating or radical-scavenging ability of tea and herbal extracts using Co(II)/EDTA-induced luminol chemiluminescence and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay (Parejo, Codina, Petrakis, & Kefalas, 2000).

3.3.1. DPPH radical method

A methanolic solution (50 μl) of the herbal extract at five different concentrations was added to 1.95 ml of DPPH· solution (6×10^{-5} M in methanol). The decrease in the absorbance at 515 nm was determined using a HP 8452A diode-array spectrophotometer until the reaction reached the steady state in the dark (Siddhuraju & Becker, 2003).

The remaining DPPH· concentration in the reaction medium was calculated from the calibration curve.

The percentage of remaining DPPH· was calculated as follows:

$$\%\ \text{DPPH}^{\cdot}\ \text{remaining} = [\text{DPPH}^{\cdot}]_T / [\text{DPPH}^{\cdot}]_{T=0},$$

where $[\text{DPPH}^{\cdot}]_T$ was the concentration of DPPH· at the time of steady state and $[\text{DPPH}^{\cdot}]_{T=0}$ was the concentration of DPPH· at zero time (Siddhuraju & Becker, 2003).

These values were plotted against mg of herbal extract/mg DPPH· to show the amount of antioxidant necessary to decrease the initial DPPH· concentration by 50% (EC_{50}) using the exponential curve.

$$[\%\ \text{DPPH}^{\cdot}\text{rem}] = b[\text{moles antioxidant/mole DPPH}^{\cdot}] + a.$$

Antiradical efficiency (AE) was also calculated ($AE = 1/EC_{50}$). Results were expressed as standard equivalents using quercetin and Trolox on the basis of the EC_{50} value.

3.3.2. Luminol chemiluminescence method

Chemiluminescence analysis was carried out on a Jenway (Essex, UK) 6200 Fluorimeter, keeping the lamp off and using only the photomultiplier of the apparatus (Parejo et al., 2000). In this method 1 ml of buffer solution (boric acid 0.05 M, pH 9), containing cobalt (II)[$CoCl_2 \cdot 6H_2O$] (2 mg/ml) and EDTA (10 mg/ml), was mixed well with 100 ml of the luminol (100 μg/ml, 5.6×10^{-4} M), buffer solution (boric acid 0.05 M, pH 9) in a test tube. Then 25 μl of H_2O_2 aqueous solution (5×10^{-5} M) was deposited on the bottom of another test tube and mixed well with 25 μl of the sample. The luminol buffer mixture was added rapidly to the cuvette with a Pasteur pipette and thoroughly mixed for 15 s in order to initiate the chemiluminescence reaction in situ. The CL intensity (I) was measured when it reached the plateau. The ratio I_0/I was calculated. I_0/I vs. μg extract/ml was plotted for three prepared dilutions of each herbal extract and a linear regression was established in order to calculate the IC_{50}, which is the amount of

sample needed to decrease by 50% the CL intensity (Parejo et al., 2000), from

$[I_0/I = a(\text{mg extract/ml}) + b]$.

The antiradical efficiency (AE) was also estimated. Results were expressed as standard equivalents using quercetin and Trolox on the basis of the IC_{50} value.

3.4. Phenolic profile determination of the herbal extracts

A Finnigan MAT Spectra System P4000 pump, coupled with a UV6000LP diode array detector and a Finnigan AQA mass spectrometer, was used for the structural elucidation of the phenolic compounds present in the herbal infusions. The separation was performed on a 125×2 mm, 4 μm, Superspher 100-4 RP-18 column (Macherey–Nagel) kept at 40 °C, at a flow rate of 0.33 ml/min, and at an injection volume of 0.5 μl (the sample solutions had an average concentration of ~50 mg extract/ml). The analysis was monitored at 290 and 340 nm and by ESI in the positive mode at a probe temperature of 450 °C, probe voltage of 4.9 kV and at 20 and 70 eV in the mass analyzer. For the gradient elution, the following programme was used: (A) H_2O (containing 2.5% AcOH); (B) MeOH:H_2O (2.5% AcOH) (6:4), isocratic at 95% A for 2 min, then 0% A in 20 min, followed by 10 min isocratic wash at 0% A. The data were processed with the Xcalibur 1.2 software.

4. Results and discussion

4.1. Determination of total phenolic content

Folin–Ciocalteu is a method used for the determination of total phenolic compounds. The content of phenolic compounds is expressed as mg gallic acid per cup of herbal infusion. The amounts of total phenolics in the studied herbs are shown in Table 1. A high content was observed in green and black teas in comparison with other herbs. Tea is known to have a high content of polyphenolics, about 36% polyphenols on a dry weight basis (Shahidi, 2000).

Table 1
The total phenolic content of the different teas and herbal infusions

Species names	Total phenolic content (mg GA/cup[a])
Greek Mountain tea, *Sideritis syriaca*	88 + 0.42[a]
Mint, *Mentha piperita*	106 + 0.18[ab]
Chamomile, *Matricaria recutita*	106 + 0.37[ab]
Dictamnus, *Origanum dictamnus*	109 + 3.20[b]
Eucalyptus *Eucalyptus globules*	113 + 1.33[b]
Sage, *Salvia fruticosa*	124 ± 1.57[b]
Linden, *Tilia* sp.	184 ± 1.72[c]
Black Ceylon tea, *Camellia sinensis*	847 ± 8.89[d]
Chinese green tea, *Camellia sinensis*	1216 ± 32.0[e]

Results are means ± S.D. ($n = 3$), $P < 0.05$; values of the same column, followed by the same letter (a–e) are not statistically different ($P < 0.05$) as measured by Duncan's test.
[a] 1 cup = 240 ml.

4.2. Evaluation of the antioxidant activity

4.2.1. DPPH free radical scavenging method

The concentration of an antioxidant needed to decrease the initial DPPH concentration by 50% (EC_{50}) is a parameter widely used to measure antioxidant activity (Sanchez, Larrauri, & Saura, 1998). Another parameter was defined as antiradical efficiency ($AE = 1/EC_{50}$) or antiradical power (ARP). The lower the EC_{50} or the higher the AE, the higher is the antioxidant activity (Brand-Williams, Cuvelier, & Berset, 1995). The scavenging activity of the herbal extracts is shown in Table 2. Green tea had the highest hydrogen-donating capacity, closely followed by black tea, while Greek mountain tea was the weakest of all. The antioxidant activity of the extracts was expressed in quercetin and Trolox equivalents by comparing EC_{50} of the herbal extracts with EC_{50} of standards. Extracts range from 0.38 QE and 0.57 TE for green tea to 0.08 QE and 0.13 TE for Greek mountain tea. One milligram of the green tea infusion is

Table 2
Estimation of free radical-scavenging activity of the herbal extracts

Herb	EC_{50}	AE	Quercetin equivalents[a]	Trolox equivalents[b]
Chinese green tea	0.15 ± 0.00[a]	6.65 ± 0.01[a]	0.38	0.57
Black tea	0.17 ± 0.00[a]	5.57 ± 0.01[b]	0.34	0.54
Dictamnus	0.23 ± 0.01[b]	4.29 ± 0.21[c]	0.25	0.4
Eucalyptus	0.24 ± 0.01[b]	4.14 ± 0.18[c]	0.24	0.38
Sage	0.35 ± 0.00[c]	2.87 ± 0.33[d]	0.18	0.27
Linden	0.35 ± 0.01[c]	2.82 ± 0.06[d]	0.17	0.26
Mint	0.46 ± 0.01[d]	2.16 ± 0.04[e]	0.13	0.21
Chamomile	0.59 ± 0.06[e]	1.01 ± 0.22[f]	0.11	0.17
Mountain tea	0.77 ± 0.01[f]	1.30 ± 0.02[g]	0.08	0.13

Results are means ± S.D. ($n = 3$), $P < 0.05$; values of the same column, followed by the same letter (a–g) are not statistically different ($P < 0.05$) as measured by Duncan's test.
[a] For quercetin $EC_{50} = 0.06$ mg quercetin/mg DPPH.
[b] For Trolox $EC_{50} = 0.096$ mg trolox/mg DPPH.

equivalent to 0.38 mg of pure quercetin in terms of DPPH radical-scavenging capacity.

4.2.2. Co(II)/EDTA-induced luminol chemiluminescence assay

The concentration of an antioxidant needed to decrease the initial chemiluminescence intensity (I_0) by 50% (IC_{50}) is a parameter used to measure antioxidant activity. For all extracts, the IC_{50} and AE were estimated in order to measure their relative hydroxyl radical-scavenging activities. The lower the IC_{50} or the higher the AE value, the higher is the antioxidant activity (Parejo et al., 2000). The highest antioxidant activity, according to the CL method, was shown by the green tea extract, closely followed by black tea while the poorest was shown by the Greek mountain tea (Table 3). The antioxidant activity of the extracts was expressed in quercetin and Trolox equivalents by comparing $IC_{50}$of the herbal extracts with $IC_{50}$of standards. Extracts range from 1.89 QE and 5.89 TE for the Chinese green tea to 0.29 QE and 0.90 TE for the Greek mountain tea.

4.2.3. Correlation of CL, DPPH and FC tests

In order to correlate these methods a regression model was used. The correlation between TPH and the antioxidant activity of the herbal extracts ($R^2 = 0.58$ for TPH/DPPH; $R^2 = 0.53$ for TPH/CL) was not highly significant. It is known that the antioxidant properties of single compounds within a group can vary remarkably so that the same levels of phenolics do not necessarily correspond to the same antioxidant responses (Zheng & Wang, 2001; Parejo et al., 2002). Moreover, the response of phenolics in the Folin–Ciocalteu assay also depends on their chemical structure, and the radical-scavenging capacity of an extract cannot be predicted on the basis of its TPH content (Parejo et al., 2000). On the other hand, a highly significant correlation coefficient ($R^2 = 0.97$) was observed between the DPPH and CL methods, which might be explained by the mechanism by which phenols scavenge the stable DPPH radical and the hydroxyl radical in these assays.

4.3. Phenolic profile of tea and herbal infusions

4.3.1. General profile

In this study the combination of diode array detection (DAD) and positive electrospray ionization mass spectrometry (ESI+), coupled to the HPLC using reverse-phase silica provided an accurate method for the structure elucidation of individual phenolics. Under the conditions used, all the compounds analyzed had an intense signal corresponding to the pseudo-molecular ion $[M + H]^+$. To a lesser extent, water adducts $[M + 18]^{+\cdot}$ and sodium adducts $[M + 23]^+$ were also demonstrated by Kiehne and Engelhardt (1996).

The MS and UV characteristics of the identified phenolics in each extract are given in Tables 4–12. About 60 different phenolic compounds were detected in the nine studied teas and herbs. Phenolic acids and their derivatives are detected in all herbal infusions while the presence of flavonoids varied: catechins were present in green tea (Table 4), black tea (Table 5) and linden (Table 7). These teas were characterized by the absence of flavanones, isoflavones, and flavones, while mint infusion was characterized by the presence of flavanones, isoflavones, and flavones (Table 7). Dictamnus contained flavanones, isoflavones, flavones and flavonols (Table 11) (Skoula & Harborne, 2002). Sage tea contains mostly flavones (Table 8) while eucalyptus tea contained only flavonols (Table 9). The only flavone detected in Greek mountain tea was apigenin 7-glycoside (Table 12).

Identification of the individual phenolic compounds was achieved by comparison of the UV–Vis absorption spectra and MS data with the literature (Fang, Yu, & Prior, 2002; Sakakibara, Honda, Nakagawa, Ashida, & Kanazawa, 2003).

Table 3
Efficient concentrations, antiradical efficiencies and standard equivalents of the herbal extract for the evaluation of Co(II)/EDTA-induced luminol chemiluminescence assay

Herb	IC_{50}	AE	Quercetin equivalents[a]	Trolox equivalents[b]
Chinese green tea	$0.17 \pm 3.40 \times 10^{-3a}$	5.94 ± 0.12^{a}	1.89	5.89
Black tea	$0.18 \pm 4.40 \times 10^{-3a}$	5.59 ± 0.14^{b}	1.78	5.56
Dictamnus	$0.25 \pm 1.07 \times 10^{-2b}$	4.00 ± 0.17^{c}	1.28	4.00
Sage	$0.28 \pm 5.78 \times 10^{-2b}$	3.54 ± 0.09^{d}	1.15	3.57
Eucalyptus	$0.35 \pm 1.54 \times 10^{-2c}$	2.84 ± 0.12^{e}	0.92	2.86
Linden	$0.50 \pm 1.66 \times 10^{-2d}$	2.00 ± 0.06^{f}	0.64	2.00
Mint	$0.59 \pm 4.31 \times 10^{-2e}$	1.71 ± 0.12^{g}	0.55	1.69
Chamomile	$0.77 \pm 2.59 \times 10^{-2f}$	1.31 ± 0.05^{h}	0.42	1.30
Mountain tea	$1.10 \pm 1.86 \times 10^{-2g}$	0.91 ± 0.01^{i}	0.29	0.90

Results are means ± S.D. ($n = 3$), $P < 0.05$; values of the same column, followed by the same letter (a–i) are not statistically different ($P < 0.05$) as measured by Duncan's test.

[a] For quercetin $IC_{50} = 0.32$ μg/ml.

[b] For Trolox $IC_{50} = 1$ μg/ml.

Table 4
LC-DAD-MS characteristics of phenols identified in the Chinese green tea infusion

Compound	RT min	λ max	$[M+H]^+$	Fragment ions	Proposed structure
1	10.37	238sh, 274	459	289, 139	Gallocatechin 3-gallate
2	10.97	242sh, 278	563	603[a] , 581[b], 291, 139	Catechin dimmer
3	11.68	270	195	–	Caffeine
4	12.73	238sh, 278	473	289, 139	Epigallocatechin 3-methyl gallate
5	13.25	238sh, 278	443	273, 153	Epicatechin 3-gallate
6	14.99	258, 358	481	319	Myricetin 3-glycoside
7	16.82	254, 354	465	487, 303	Quercetin 3-glycoside
8	17.09	254, 354	465	487, 303	Quercetin 3-glycoside
9	18.27	238sh, 262, 346	449	471, 287	Kaempferol 3-glycoside
10	18.83	262, 346	595	471, 449, 287	Kaempferol 3-rutinoside
11	21.66	238, 266sh	565	467, 181, 163	Ester of caffeic acid
12	22.98	262, 298	497	165, 147	Ester of coumaric acid
13	31.01	262	197	–	Xanthoxylin

[a] $[M+Na]^+$.
[b] $[M+H_2O]^{+\cdot}$.

Table 5
LC-DAD-MS characteristics of phenols identified in the black tea infusion

Compound	RT min	λ max	$[M+H]^+$	Fragment ions	Proposed structure
1	2.10	270	295	171, 153	Ester of gallic acid
2	6.10	274	579	153	Ester of gallic acid
3	6.66	270	181	–	Theobromine
4	7.72	242, 278	291	139	Catechin
5	9.05	242sh, 274	333	153, 163	Ester between caffeic and gallic acid
6	10.38	238sh, 274	459	289, 139	Gallocatechin 3-gallate
7	10.95	238sh, 278	291	139	Catechin
8	11.52	258	195	–	Caffeine
9	12.70	310	467	275, 153	Ester between epiafzelechin and gallic acid
10	13.23	238sh, 274	443	273, 153	Catechin gallate
11	14.07	238, 278	611	633[a], 441, 289, 153	Epicatechin di-gallate
12	14.98	264, 354	481	503[a], 319	Myricetin 3-glycoside
13	15.97	278, 346sh	595	617[a], 612[b], 319, 153	Myricetin gallolyl glycoside
14	17.05	254, 302sh, 354	611	487, 465, 303	Quercetin 3-diglycoside
15	18.58	254, 350	449	471[a], 303	Quercetin 3- glycoside
16	18.78	262, 350	595	617[a], 287	Kaempferol 3- rutinoside
17	20.53	262, 346	433	455[a], 287	Kaempferol 3-glycoside
18	21.28	270, 374, 454sh	579	153, 139	Procyanidin B2
19	21.80	274, 374, 454	717	565, 139, 153	Theaflavin 3-gallate
20	22.70	266, 314	757	477, 455, 303, 147	Quercetin dicoumaryl glycoside
21	24,00	266, 318	741	477, 455, 287, 147	Kaempferol dicoumaryl glycoside
22	30.96	262	197	–	Xanthoxylin

[a] $[M+Na]^+$.
[b] $[M+H_2O]^+$.

4.4. Identification of phenolic acids and their derivatives

The spectra generated for benzoic and cinnamic acid derivatives gave their molecular ion and their characteristic aroyl (benzoyl, cinnamoyl) fragments due to the losses of OH or OR groups. Rosmarinic acid, expected in sage, was not found in the present study and this could be due to the fact that ethyl acetate, known as a medium polarity solvent, was used for the recovery of the phenolics.

4.4.1. Identification of flavonoids

Most of the flavonoids detected in this study were glycosides, their mass spectra showing both the protonated molecule $[M+H]^+$ and the ion corresponding to the protonated aglycone $[A+H]^+$. The latter is formed by loss of the glucose, galactose, rhamnose and xylose moieties from the glycosides.

UV/Vis spectra have long been used for structural analysis of flavonoids. The typical flavonoid spectrum consists of two maxima in the range 240–285 nm (Band II) determined by the A ring, and 300–550 nm (Band I), which is more specific and useful for obtaining information regarding identification. The position and relative intensities of these maxima yield information on the nature of the flavonoid and its oxygenation pattern; variation within these ranges will depend on the hydroxylation pattern and on the degree of substitution of

Table 6
LC-DAD-MS characteristics of phenols identified in the chamomile infusion

Compound	RT min	λ max	$[M+H]^+$	Fragment ions	Proposed structure
1	2.78	282	269	131	Ester of cinnamic acid
2	7.97	234sh, 314	325	163	Ester of caffeic acid
3	9.52	246, 318	355	163	Chlorogenic acid
4	11.09	242, 302	379	195, 177	Ester of ferulic acid
5	12.77	318	163	–	Methoxycinamaldehyde
6	14.16	258, 267sh, 358	481	319	Myricetin 3-glycoside
7	14.67	242, 294sh, 318	379	195, 177	Ester of ferulic acid
8	15.62	254, 370	465	303	Quercetin 7-glycoside
9	15.82	246, 322	517	539[a], 163	Ester of caffeic acid
10	16.62	254, 346	449	287	Luteolin 7-glycoside
11	16.86	258, 358	495	333, 318	Patuletin 7-glycoside
12	17.68	242, 294sh, 330	509	195, 177	Ester of ferulic acid
13	17.93	246, 298sh, 330	643	517, 163	Ester of caffeic acid
14	18.27	262, 318sh, 342	433	271	Apigenin 7-glycoside
15	18.92	254, 318	509	332, 177	Ester of ferulic acid
16	19.48	242, 318	519	541[a], 325, 163	Ester of caffeic acid
17	20.50	254, 334	519	271	Apigenin 7-(6″ malonyl glycoside)
18	21.58	242, 318	519	541[a], 177, 163	Ester between caffeic and ferulic acid
19	22.17	266, 338	561	271	Apigenin 7-apiosylglycoside
20	22.65	266, 334	475	271	Apigenin 7-(6″ acetyl glycoside)
21	23.66	266, 334	561	271	Isomer of 21
22	30.95	262	197	–	Xanthoxylin

[a] $[M+Na]^+$.

Table 7
LC-DAD-MS characteristics of phenols identified in the linden infusion

Compound	RT min	λ max	$[M+H]^+$	Fragment ions	Proposed structure
1	3.85	258, 294sh	139	–	*p*-Hydroxybenzoic acid
2	6.72	254, 333sh	579	339, 139	Procyanidin B4
3	7.53	278	291	139	Catechin
4	9.04	242, 314	349	165, 147	Ester of coumaric acid
5	10.98	242, 278	563	598[a], 291, 139	Catechin dimmer
6	14.31	242, 318	563	195, 177	Ester of ferulic acid
7	15.01	294	333	165	Eugenol ester
8	15.90	254, 354	611	303	Quercetin 3-glycoside 7-rhamnoside
9	17.20	254, 318sh, 354	465	303	Quercetin glycoside
10	18.75	254, 350	595	449, 303	Quercetin 3,7-dirhamnoside
11	18.95	262, 318sh, 346	449	471[a], 287	Kaempferol 3-glycoside
12	20.74	262, 342	433	455[a], 287	Kaempferol 3-rhamnoside
13	22.67	266, 314	595	287, 147	Tiliroside
14	31.81	262	197	-	Xanthoxylin

[a] $[M+Na]^+$.

the hydroxyls (Merken & Gray, 2000; Santos-Buelga, Garcia-Viguera, & Tomas-Barberan, 2003). For example, quercetin 7-glycoside had λ max: 254 and 370 nm, while quercetin 3-glycoside had λ max: 254 and 354 nm. The latter shows a hypsochromic shift of 16 nm due to the glycosidation at the C_3 position whereas the former does not show this effect, having the same spectrum as the aglycone. It is known that the introduction of a glycoside on the hydroxyls at positions 7, 3 or 4 has no effect on the wavelength maximum or the spectrum shape (Santos-Buelga et al., 2003). Kaempferol 3-glycoside and luteolin 7-glycoside yielded the same fragment at m/z 287, which represents their corresponding aglycone, but could be differentiated on the basis of the UV/Vis absorption due to the B rings, where the former has a Band II peak at 264 nm while luteolin 7-glycoside has one at 254 nm. This fact could be explained by the difference in the positions of OH groups between these flavonoids (Santos-Buelga et al., 2003). Moreover, apigenin and genistein, detected in mint infusion, also having the same molecular weight, were identified on the basis of their UV spectra. The UV spectrum of apigenin 7-glycoside is characterized by the presence of two maxima at 262 and 333 nm, while the spectrum of genistein consists of a prominent band at 286 nm with a shoulder in the 350 nm (Band II) region. Finally, flavanones exhibit a very strong maximum at 285 nm (Band II) and a small peak or shoulder at 320–330 nm (Band I).

Table 8
LC-DAD-MS characteristics of phenols identified in the sage infusion

Compound	RT min	λ max	$[M+H]^+$	Fragment ions	Proposed structure
1	3.48	274	169	151	Vanillic acid
2	6.12	278, 310sh	139	–	*p*-Hydroxybenzoic acid
3	9.65	242, 318	181	163	Caffeic acid
4	11.32	238sh, 278	345	137	Ester of gentisic acid
5	12.78	238sh, 310	335	227, 165, 151	Ester of vanillic acid
6	16.76	254, 342	595	449, 287	Luteolin 7-diglycoside
7	17.10	254, 358	465	487[a], 303	Quercetin 3-glycoside
8	17.65	246, 330	361	378[b], 163	Ester of caffeic acid
9	18.31	266, 338	433	271	Apigenin 7-glycoside
10	18.98	270, 334	463	301, 286	Hispidulin 7-glycoside
11	21.71	254, 294sh, 350	577	287	Luteolin 7-glycoside
12	23.70	266, 342	565	271	Apigenin 7-glycoside
13	24.00	270, 338	301	286, 200	Hispidulin
14	25.55	274, 334	315	337[a], 271, 254	Cirsimatrin
15	30.85	262	197	–	Xantoxylin
16	33.13	274, 334	329	351[a], 268, 168	Salvigenin
17	35.06	242, 282	331	353[a], 285, 215	Cirsiliol

[a] $[M+Na]^+$.
[b] $[M+H_2O]^+$.

Table 9
LC-DAD-MS characteristics of phenols identified in the eucalyptus infusion

Compound	RT min	λ max	$[M+H]^+$	Fragment ions	Proposed structure
1	2.08	270	277	153	Ester of gallic acid
2	7.23	242, 278sh	323	344[a], 339[b], 147	Ester of *o*-coumaric acid
3	9.93	254, 294sh	355	204, 151	Ester of vanillic acid
4	11.13	270, 374sh	211	153	Ester of gallic acid
5	12.49	306	351	335, 151	Ester of vanillic acid
6	16.61	254, 358	465	487[a], 303	Quercetin 3-glycoside
7	17.57	254, 354	585	629[c], 435, 303, 169, 151	Quercetin vanillin glycoside
8	18.07	266, 350	449	471[a], 287	Kaempferol 3-glycoside
9	18.97	266, 346	419	441[a], 287	Kaempferol 3-rhamnoside
10	22.15	266, 290	631	303, 177, 149	Ester of ellagic acid
12	30.75	262	197	–	Xantoxylin

[a] $[M+Na]^+$.
[b] $[M+H_2O]^+$.
[c] [M + MeOH].

Table 10
LC-DAD-MS characteristics of phenols identified in the mint infusion

Compound	RT min	λ max	$[M+H]^+$	Fragment ions	Proposed structure
1	9.10	242, 318	181	163	Caffeic acid
2	12.85	306	227	–	Dibenzoic acid
3	14.73	282, 330sh	597	289	Eriodictyol 7-rutinoside
4	16.97	254, 350	595	287	Luteolin 7-rutinoside
5	17.87	246, 314, 334sh	611	378, 163	Ester of caffeic acid
6	18.49	266, 338	579	271	Apigenin 7-rutinoside
7	18.72	286	271	289	Genistein
8	20.96	238, 314	295	147	Ester of coumaric acid
9	26.67	250, 290, 346	375	397[a], 345, 330	Gardenin D
10	31.70	262	197	–	Xantoxylin
11	40.47	282, 334	359	329, 286	Gardenin B

[a] $[M+Na]^+$.

Table 11
LC-DAD-MS characteristics of phenols identified in the dictamnus infusion

Compound	RT min	λ max	$[M+H]^+$	Fragment ions	Proposed structure
1	3.46	238, 270	169	151	Vanillic acid
2	6.07	278, 310sh	139	–	*p*-Hydroxybenzoic acid
3	8.69	290	165	–	Eugenol
4	9.63	242, 318	181	163	Caffeic acid
5	12.80	238, 310	227	165, 147	Ester of coumaric acid
6	13.55	238, 290	305	153	Ester of gallic acid
7	14.38	290, 325sh	285	–	Biochanin A
8	15.00	286, 330sh	611	305	Taxifolin glycoside
9	16.80	258, 338	465	487[a], 303, 287	Luteolin Me-glycoside
10	17.73	242, 330	379	361, 163	Ester of caffeic acid
11	18.59	290, 330sh	289		Eriodictyol
12	19.43	238, 286, 314sh	595	617[a], 289, 147	Ester of coumaric acid
13	23.88	266, 298sh, 338	565	271	Apigenin 6,8-di-glycoside
14	25.97	270, 346	345	367[a], 284, 269	Penduletin
15	27.81	282, 344	345	315, 297, 272	Dihydroxy trimethoxy flavone
16	31.15	262	197	–	Xanthoxylin

[a] $[M+Na]^+$.

Table 12
LC-DAD-MS characteristics of phenols identified in the Greek mountain tea infusion

Compound	RT min	λ max	$[M+H]^+$	Fragment ions	Proposed structure
1	9.14	246, 298, 322	355	163	Chlorogenic acid
2	14.07	242, 322	263	285[a], 280[b], 195, 177	Ester of ferulic acid
3	15.69	246, 330	643	325, 163	Ester of caffeic acid
4	16.15	246, 330	325	163	Ester of caffeic acid
5	17.03	242, 330	643	325, 163	Ester of caffeic acid (isomer of **3**)
6	17.43	242, 330	639	339, 177	Ester of ferulic acid
7	18.19	266, 338	433	271	Apigenin 7-xyloside
8	21.38	238, 318	595	301, 177	Ester of ferulic acid
9	22.98	238, 270sh, 318	581	603[a], 413, 273, 147	Ester of coumaric acid

[a] $[M+Na]^+$.
[b] $[M+H2O]^+$.

4.4.2. Acylated glycosides

In this study, acylated flavonol glycosides with aliphatic (acetic, malic) or aromatic acids (coumaric, ferulic, gallic) were observed as expected (Duke, 2000; Harborne & Baxter, 1999). Acylation on the sugar residue always affects the retention time by generally increasing it, while spectra of the acylated glycosides are influenced by the nature of the acyl residue. It is assumed that flavonoids acylated with aliphatic acids display the same spectra as the non-acylated ones (Santos-Buelga et al., 2003). This effect is observed for apigenin acetyl glycoside (λ max: 266–333 nm), detected in chamomile infusion (Table 6), while acylation with hydroxycinamic acids makes Band I of the spectrum shift to a lower wavelength with an increase in intensity depending on the number of the acyl residues present in the molecule (Santos-Buelga et al., 2003). A shoulder or a peak appears in the spectrum of the flavonols at 305–310 nm, characteristic of this type of residue. This effect is observed for tiliroside, detected in linden (Table 7) and for quercetin dicoumaryl glycoside detected in black tea. Acylation with benzoic acid induces a shift of the absorption of band II to 260–290 nm, which is the maximum absorption observed for myricetin galloyl glycoside, detected in black tea (Table 6).

4.4.3. Identification of catechins

The UV spectra of catechins shows maximum absorption at non-specific wavelengths (270–290 nm), at which many phenolics also absorb, thus not permitting their selective detection and identification (Merken & Gray, 2000; Santos-Buelga et al., 2003). Therefore, their identity was confirmed on the basis of the mass spectra obtained. A typical mass spectrum, obtained from LC–MS analysis, exhibits a strong protonated molecular ion of the catechin at m/z 291 and its characteristic fragment at m/z 139, which corresponds to the A ring fragment produced by a retro Diels–Alder reaction (Zeeb, Nelson, Albert, & Dalluge, 2000).

Among the identified catechins were: catechin detected in black tea (Table 5) and linden (Table 7) (m/z 291 → m/z 139); gallocatechin 3-gallate detected in black tea (Table 5) and green tea (Table 4), which showed the presence of $[M+1]^+$ at m/z 459 and the characteristic fragment ions at m/z 289 $[M+H–galloyl+H–H_2O]^+$ and m/z 139, and epigallocatechin 3-methyl gallate detected

in green tea (Table 4). The observation that the latter had a mass shift of 14 amu relative to gallocatechin 3-gallate led to the tentative assignment of this compound (Zeeb et al., 2000). This assignment is supported by the presence of m/z 139, suggesting that the compound is structurally related to a catechin and the presence of m/z 289, suggesting the relationship of this compound to epigallocatechin gallate and indicating that the A and B rings are both unmodified, leaving the possibility only of a methylated gallic acid moiety (Zeeb et al., 2000). Other identified catechins were: epicatechin 3-gallate detected in green tea: (m/z 443 → m/z 273 [M + H–galloyl + H–H_2O]$^+$ and m/z at 153, corresponding to a galloyl moiety, Table 4) (Zeeb et al., 2000), epicatechin digallate detected in black tea (m/z 611 → m/z 289 [M + H–2 galloyl + H–H_2O]$^+$ and m/z 153, Table 5) and a catechin dimer detected in green tea (Table 5) and linden (Table 7), which is characterized by the molecular ion at m/z 563 and the fragments at m/z 291 (catechin ion) and m/z 139 (catechin characteristic fragment).

An ester between epiafzelchin and gallic acid, detected in black tea (Table 5), yielded a molecular ion at m/z 467 and showed the fragmentation pattern m/z 275 (M + 1 of epiafzelchin) and m/z 153 (galloyl moiety). The presence of epiafzelchin in tea has previously been reported (Zeeb et al., 2000).

Black tea catechins are subjected to polymerization due to the manufacturing process (Wang, Provan, & Helliwell, 2001). Among the catechin polymers, procyanidin B2 (m/z 579 → m/z 139) and m/z 153 (galloyl moiety) and theaflavin 3-gallate [m/z 717 → m/z 565 (M + H–galloyl) → m/z 139 and m/z 153 (galloyl moiety)] were detected.

4.4.4. Caffeine

Caffeine was detected in tea as expected. The UV and mass spectra of caffeine, detected in green tea and black tea (Tables 4 and 5) and theobromine, detected in black tea (Table 5), was consistent with literature values.

Regarding the important place that tea and herbal infusions have as a popular beverages in the Mediterranean region and the increased interest in recent years for food and beverages enriched in beneficial health constituents, it may be suggested that tea and herbal infusions can be major sources of polyphenols that exhibit important antioxidant behavior.

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