Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 110 (2008) 718–728
FOOD CHEMISTRY
www.elsevier.com/locate/foodchem

Analytical Methods

Cymbopogon citratus leaves: Characterisation of flavonoids by HPLC–PDA–ESI/MS/MS and an approach to their potential as a source of bioactive polyphenols

Artur Figueirinha [a,d], António Paranhos [a,b], José J. Pérez-Alonso [c], Celestino Santos-Buelga [c], Maria T. Batista [a,b,*]

[a] *Centro de Estudos Farmacêuticos, Faculdade de Farmácia, Universidade de Coimbra, 3000-295 Coimbra, Portugal*
[b] *Laboratório de Farmacognosia, Faculdade de Farmácia, Universidade de Coimbra, 3000-295 Coimbra, Portugal*
[c] *Unidad de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Salamanca, 37007 Salamanca, Spain*
[d] *Departamento de Ambiente, Instituto Politécnico de Viseu, Campus Politécnico de Repeses, 3504-510 Viseu, Portugal*

Received 15 June 2007; received in revised form 23 November 2007; accepted 14 February 2008

Abstract

Leaves from cultivated *Cymbopogon citratus* were extracted with methanol, 80% aqueous ethanol and water (infusion and decoction) and the extracts were assessed for their antiradical capacity by 2,2-diphenyl-1-picrylhydrazyl (DPPH•) assay; the infusion extract exhibited the strongest activity. Tannins, phenolic acids (caffeic and *p*-coumaric acid derivatives) and flavone glycosides (apigenin and luteolin derivatives) were identified in three different fractions obtained from an essential oil-free infusion, and a correlation with their scavenger capacity for reactive oxygen species was studied. The tannin and flavonoid fractions were the most active against species involved in oxidative damage processes. In the flavonoid fraction, representing 6.1% of the extract, thirteen compounds (*O*- and *C*-glycosylflavones) were tentatively identified by high performance liquid chromatography, coupled to photodiode-array and electrospray ionization mass spectrometry detectors (HPLC–PDA–ESI/MS), nine of which were identified for the first time in this plant, all of them being *C*-glycosylflavones (mono-*C*-, di-*C*- and *O*,*C*-diglycosylflavones). The potential beneficial and protective value of the identified polyphenols for human health is discussed.

Keywords: *Cymbopogon citratus* leaves; Lemon grass; *Gramineae*; Free radical-scavenging; Superoxide anion; Hydroxyl radical; *O*- and *C*-Glycosylflavones; HPLC–PDA–ESI/MS^n; Polyphenol fractionation; Flavonoid isolation

1. Introduction

Cymbopogon citratus (DC) Stapf. (lemon grass), Poaceae – *Gramineae*, is a native herb from India and is also cultivated in other tropical and subtropical countries. It is consumed as an aromatic drink and used in traditional cuisine for its lemon flavour, but it is also employed in popular medicine. Infusions or decoctions of dry leaves have been utilized as stomachic, antispasmodic, carminative and antihypertensive agents (Borrelli & Izzo, 2000; Gupta, 1995). In many countries it is used to treat feverish conditions and as a relaxant and sleeping aid. It helps with emotional states and it is an antidepressant agent (Gupta, 1995). Studies on extracts from *C. citratus* leaves have demonstrated anti-inflammatory, hypotensive, vasorelaxating and diuretic activities (Carbajal, Casaco, Arruzazabala, Gonzalez, & Tolon, 1989; Runnie, Salleh, Mohamed, Head, & Abeywardena, 2004), efficiency against oxidative damage (Cápiro, Sánchez-Lamar, Fonseca, Baluja, & Borges, 2001; Melo et al., 2001) and also cancer chemopreventive properties (Puatanachokchai et al., 2002). Because *C.*

* Corresponding author. Address: Centro de Estudos Farmacêuticos, Faculdade de Farmácia, Universidade de Coimbra, 3000-295 Coimbra, Portugal. Tel.: +351 239 859995; fax: +351 239 827126.
E-mail address: mtpmb@ff.uc.pt (M.T. Batista).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.02.045

citratus leaves constitute a source of essential oil for the flavour and fragrance industries, most uses and phytochemical studies are centred on their volatile compounds (Baratta et al., 1998; Kasali, Oyedeji, & Ashilokun, 2001). However, many of the activities previously described have been attributed to polyphenols, which have gained considerable attention, particularly because of their antioxidant and radical-scavenging activities. Epidemiological studies have underlined the protective role of polyphenols against degenerative and cardiovascular diseases, and the possibility of incorporating these compounds in functional foods has been the object of growing interest.

Preliminary results obtained by us (Figueirinha, Cruz, Lopes, & Batista, 2006) proved that an essential oil-free infusion from *C. citratus* leaves induced a reduction of the nitric oxide (NO) production in dendritic cells coincubated with an inflammatory stimulus (lipopolysaccharide – LPS), allowing cell viability under the assayed conditions.

The presented research concerns the fractionation of phenolic compounds from an essential oil-free infusion of a cultivated plant, and the establishment of the profile of the flavonoids by spectrometric techniques (ultra-violet and mass spectrometry) coupled with high performance liquid chromatography. The relationship between the flavonoid structures and their protective effects against reactive oxygen species (ROS), inflammatory and/or degenerative diseases will also be considered.

2. Materials and methods

2.1. Chemicals, standards and enzymes

Aluminium chloride ($AlCl_3$), sodium acetate anhydrous (NaOAc), boric acid (H_3BO_3), sodium hydroxide (NaOH), anhydrous ferric chloride ($FeCl_3$), hydrogen peroxide (perhydrol® 30%) and trichloroacetic acid were purchased from Merck (Darmstadt, Germany). Hydrochloric acid (HCl) from J.T. Baker (Deventer, Holland) and 2,2-diphenyl-1-picrylhydrazyl (DPPH•), ascorbic acid, 2-deoxy-D-ribose, ethylenediaminetetraacetic acid (EDTA) disodium salt, *p*-nitro blue tetrazolium chloride (NBT), riboflavin, superoxide dismutase (SOD), N,N,N′,N′-tetramethylethylenediamine (TEMED) and 2-thiobarbituric acid (TBA) were purchased from Sigma Chemical (St. Louis, MO). The reference compounds used were: D(−) ribose (Merck – Darmstadt, Germany), L(+) rhamnose and D(+) xylose (Fluka – Buchs, Switzerland), β-D(+) glucose, D(+) galactose, D(−) arabinose, chlorogenic acid, caffeic acid and *p*-coumaric acid (Sigma Chemical – St. Louis, MO), luteolin, isovitexin, orientin, isoorientin, 3′,7-di-*O*-glucosyl luteolin, 4′-*O*-glucosyl luteolin and 7-*O*-glucosyl luteolin (Extrasynthese – Genay, France).

High performance liquid chromatography (HPLC) grade solvents, Merck, and ultra pure Milli-Q water Millipore (Molsheim, France) were always used for HPLC and spectroscopic analyses. Analytical grade solvents from Merck (Darmstadt, Germany) were used for fractionation, isolation, thin-layer chromatography (TLC) and paper chromatography.

2.2. Plant material and preparation of the extracts

Dry leaves of *C. citratus* (DC) Stapf. were purchased from ERVITAL® (Mezio, Castro D'Aire, Portugal). The plant was cultivated in the region of Mezio, Castro D'Aire (Portugal) in a greenhouse at 1000 m above sea level. A voucher specimen is deposited in the herbarium of the University of Coimbra, Faculty of Pharmacy. The identity of the plant was confirmed by Dr. J. Paiva (Botany Department, University of Coimbra, Portugal).

Four extracts were prepared from the powdered plant material, for an antiradical activity screening. Three different extractive solvents were used: methanol at 60 °C for 5 min, 80% aqueous ethanol at room temperature using an Ultra-Turrax homogenizer for 5 min at 8000–9500 rpm and boiling water for both the decoction and infusion preparation. All extracts were prepared by adding 150 ml of solvent to 5 g of the powdered plant material. Decoction was obtained by boiling the plant material in water until the volume of water was reduced to 100 ml. Infusion was prepared by adding boiling water to the plant material, the mixture being kept hot and left to stand for 15 min. After extractions, the extracts were filtered under vacuum and their volumes made up to 150 ml with the respective extractive solvent.

As the infusion extract showed the strongest antioxidant activity, an essential oil-free infusion was subsequently prepared from it. So, an infusion obtained, as described above, was repeatedly washed with *n*-hexane to remove the less polar compounds. The aqueous phase was concentrated on a rotatory evaporator to a small volume and then freeze-dried. All subsequent study was performed with this essential oil-free infusion that will henceforth be referred to as infusion.

2.3. Infusion fractionation

Water (11.3 ml) was added to the infusion (230 mg). After centrifugation, the aqueous solution was fractionated on a reverse phase cartridge Chromabond® C18 10 g/70 ml (Macherey-Nagel, Düren, Germany), eluting with water (20 ml), giving fraction F_1 and aqueous methanol solutions, then 5% methanol (15 ml), 15% methanol (13 ml), 25% methanol (11.3 ml), 50% methanol (10.4 and 30 ml) and 80% methanol (40 ml), giving fractions F_2, F_3, F_4, F_5, F_6 and F_7, respectively. Dry residue of F_7 (54.3 mg) was recovered in 50% aqueous ethanol (3.5 ml) and fractionated by gel chromatography on a Sephadex® LH-20 (Sigma–Aldrich – Amersham, Sweden) column (88 × 2.5 cm), using ethanol as the mobile phase. Two different sub-fractions were obtained from F_7: sub-fraction F_{7a} (containing phenolic acids) and sub-fraction F_{7b} (containing flavonoids). The fractionation process described (Fig. 1) was monitored by TLC and HPLC, providing three major fractions: FI corresponds to F_6; FII comes from joining fraction F_2 and sub-

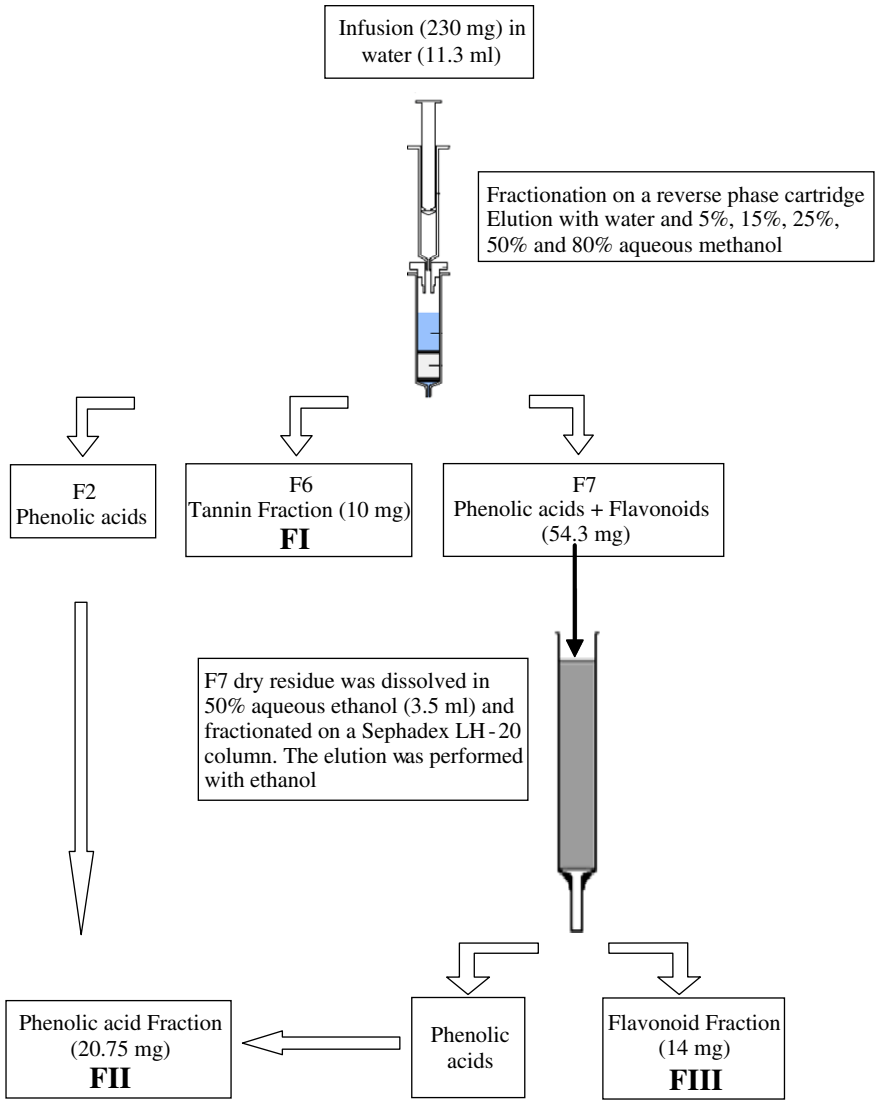


Fig. 1. Scheme of the process used for the fractionation of the *Cymbopogon citratus* leaves infusion.

fraction F_{7a}; FIII corresponds to sub-fraction F_{7b}. The fractions were taken to dryness under reduced pressure (40 °C) and weighed.

2.4. Isolation of flavonoids

Freeze-dried infusion (2 g) was treated with water (100 ml) and centrifuged. The supernatant was fractionated on a reverse phase semi-preparative column, Lichroprep® RP – 18; 310 × 25 mm (40–63 μm), Merck (Darmstadt, Germany). The elution was performed using water (100 ml) and aqueous methanol solutions with 5% methanol (201 ml), 15% methanol (115 ml), 25% methanol (82 ml), 50% methanol (370 ml) and 80% methanol (100 ml). Sixty two fractions were obtained by monitoring the eluted with an LKB BROMA 2151 UV detector (Sweden) set at 350 nm. All the fractions were analysed by TLC and HPLC and were grouped into 27 major fractions according to their composition.

Fractions containing flavonoids were dried under vacuum at 40 °C, and the residues were dissolved in methanol. The methanolic solutions from flavonoid fractions were applied on sheets of Whatman 3 MM chromatography paper, Whatman® (Maldstone, England), and were run with 15% aqueous acetic acid. The bands corresponding to the flavonoids were removed after long wave UV light (366 nm) detection and extracted with 50% aqueous methanol. The solutions were concentrated under vacuum at 40 °C.

Seven flavonoids were isolated (compounds **1**, **2**, **5**, **8**, **9**, **12** and **13**). Their purity was screened by TLC systems (S2 to S7) and HPLC. It was higher than 97% (measured by HPLC) for all flavonoids.

2.5. Thin-layer chromatography

Seven chromatographic systems were used for the TLC studies; one system for the sugars (S1) and six for the flavo-

noids (S2 to S7). The first system (S1) employed pre-coated Kieselguhr G plates, Merck (Darmstadt, Germany) impregnated with a 1.6% aqueous solution of monosodium phosphate, and a mobile phase consisting of 1.6% aqueous solution of monosodium phosphate–butanol–acetone (10:40:50). Sugars were detected by an anisaldehyde–sulphuric acid reagent (Krebs, Heusser, & Wimmer, 1969), a freshly prepared mixture of ethanol (9 ml), concentrated sulphuric acid (0.5 ml), acetic acid (0.1 ml) and anisaldehyde (0.5 ml). After spraying, the plates were heated to 90–100 °C for 5–10 min.

TLC for flavonoid analysis was carried out with six systems: S2 – pre-coated silica gel 60 F_{254} plates, Merck, were eluted with ethyl acetate–formic acid–acetic acid–water (100:11:11:26). Detection was done by spraying with a 1% solution of diphenylboric acid 2-aminoethyl ester (NP) (Sigma Chemical) in methanol, followed by 5% solution of polyethyleneglycol 400 (PEG) in ethanol (Wagner, Bladt, & Zgainski, 1984). S3 – pre-coated silica gel 60 F_{254} plates, Merck, were run with ethyl acetate–methanol–water (63:12:9). Detection was done by examining plates under UV light (366 nm) with and without ammonia fuming. The last four systems employed pre-coated cellulose MN300 plates, Macherey-Nagel (Düren, Germany), which were eluted with *n*-butanol–acetic acid–water (60:15:75) (S4), acetic acid–water (2:98) (S5), acetic acid–water (15:85) (S6) or chloroform–acetic acid–water (50:45:5) (S7). Detection under UV light (366 nm), with and without ammonia fuming, was used for these four systems.

2.6. HPLC–PDA

HPLC analysis was performed in a Gilson apparatus equipped with a photodiode-array detector (PDA), Gilson Electronics SA (Villiers le Bel, France). The studies were carried out on a Spherisorb S5 ODS-2 column (250 × 4.6 mm i.d., 5 μm), Waters Corporation (Milford, Massachusetts) and a Nucleosil guard cartridge C18 (30 × 4 mm i.d., 5 μm), Macherey-Nagel (Düren, Germany), at 24 °C. A mobile phase, consisting of 5% aqueous formic acid v/v (A) and methanol (B), was used with a discontinuous gradient of 5–15% B (0–10 min), 15–30% B (10–15 min), 30–35% B (15–25 min), 35–50% B (25–35 min), 50–80% B (35–40 min), followed by an isocratic elution during 20 min, at a flow rate of 1 ml min^{-1}. Chromatographic profiles were acquired in the wavelength range 200–600 nm. Data treatment was carried out with software Unipoint® 2.10 Gilson. The compound classes were identified by their UV spectra and retention times, comparing them with standards.

Quantification of the flavonoids was performed by the absorbance recorded in the chromatograms relative to two external standards (isoorientin and vitexin). Luteolin and apigenin glycosides were quantified as isoorientin (at 350 nm) and as vitexin (at 335 nm), respectively. The correlation between peak area and concentration was assessed by the least-squares regression model. The identification of the flavonoids, to quantify, was done by comparing their UV spectra and retention times, with either those of commercial standards or with those of the flavonoids that were isolated in this work, after they had been identified by HPLC–PDA–ESI/MS^n.

2.7. HPLC–PDA–ESI/MS^n

Flavonoid analyses from FIII and from isolated flavonoids were carried out on an HP 1100 liquid chromatograph system equipped with a PDA detector (Agilent Technologies, Waldbronn, Germany) and interfaced with a Finnigan LCQ (San Jose, CA) mass spectrometer equipped with an API-ES ionization chamber. Separation was performed on an AQUA® C18 reverse phase column, 150 × 46 mm 5 μm (Phenomenex, Torrance, CA, USA) at 25 °C, using 2.5% aqueous acetic acid (A), 2.5% aqueous acetic acid–acetonitrile 90:10 (B) and acetonitrile (C) as mobile phase. The gradient profile used was 0–100% B in A (0–5 min), 0–15% C in B (5–30 min), 15–50% C in B (30–35 min) and 50% C in B (35–40 min) isocratically, at a flow rate of 0.5 ml min^{-1}. The first detection was done with a PDA detector in a wavelength range 250–600 nm, followed by a second detection in the mass spectrometer.

Mass analyses were obtained in the negative ion mode. The mass spectrometer was programmed to perform three consecutive scans: full mass (*m*/*z* 125–1500), MS^2 of the most abundant ion in the full mass and MS^3 of the most abundant ion in the MS^2. Source voltage was 2.5 kV and the capillary voltage and temperature were −10 V and 175 °C. Nitrogen was used as sheath and auxiliary gas at flow rates of 1.2 and 6 l/min, respectively. The normalised energy of collision was 45%, using helium as collision gas.

2.8. Acid hydrolysis

Aliquots (2 mg) from dry residues of each of the isolated flavonoids were dissolved in methanol–water, 1:1 (2 ml) and heated, under nitrogen (N_2) at 100 °C after addition of 2 M aqueous HCl (2 ml). After 1 h, the samples were cooled, water (2 ml) was added and the solution was concentrated under vacuum at 40 °C. Water addition and concentration were repeated until elimination of all acid was achieved. The aqueous solution was then successively fractionated by liquid–liquid extraction with ethyl ether, ethyl acetate and *n*-butanol (3 × 5 ml). Each of these fractions was analysed by HPLC–PDA. The identification of the sugars was done in the remaining aqueous solution, using system S1 and different sugars as standards.

2.9. UV spectral shifts

Aliquots of the isolated flavonoids were dissolved in an appropriate volume of methanol. Ultraviolet spectra in methanol and in methanolic 1 M NaOH, $AlCl_3$, $AlCl_3$ + HCl, NaOAc and NaOAc + H_3BO_3 were recorded

according to Mabry, Markham, and Thomas (1970). All the measurements were made against a blank prepared, as for the isolated compounds.

2.10. Antioxidant assessment

2.10.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical assay

Free radical-scavenging activity was evaluated according to the method described by Blois (1958). Aliquots of samples (100 μl) were assessed by their reactivity with methanolic solution of 500 μM DPPH (500 μl) in the presence of 100 mM acetate buffer, pH 6.0 (1 ml). The reaction mixtures (3 ml) were kept for 30 min at room temperature and in the dark. The decreases in the absorbance were measured at 517 nm, in a Hitachi U-2000 (Tokyo, Japan) spectrophotometer.

2.10.2. Superoxide anion-scavenging assay

The assay was based on the superoxide-driven reduction of NBT by photochemically reduced riboflavin (Kostyuk, Potapovich, Vladykovskaya, & Hiramatsu, 2000). Reaction mixtures (3 ml) contained phosphate buffer (16 mM, pH 7.8), EDTA (0.1 mM), TEMED (0.8 mM), NBT (85 μM), riboflavin (6 μM) and the appropriate volume of the samples. The assay was carried out at room temperature (22 °C) under fluorescent lighting (20 W, 20 cm). Reaction was stopped by switching off the light and the addition of 50 μl SOD (1 mg ml^{-1}). Absorbance was measured at 560 nm.

2.10.3. Hydroxyl radical-scavenging assay

The scavenging capacity for hydroxyl radical was evaluated using a deoxyribose degradation assay performed according to the procedure described by Payá, Halliwell, and Hoult (1992). In a final volume of 1 ml, reaction mixtures contained 20 mM phosphate buffer (pH 7.4), 2.8 mM 2-deoxy-D-ribose, 104 μM EDTA, 20 μM $FeCl_3$, 0.5 mM H_2O_2, 0.1 mM ascorbic acid, and the sample aliquots. Mixtures were kept at 37 °C for 1 h, and then the extent of deoxyribose degradation was measured by adding 1 ml of 1% thiobarbituric acid (w/v) and 1 ml of 2.8% trichloroacetic acid (w/v), followed by heating at 100 °C for 20 min. After cooling, the absorbance at 532 nm was measured.

3. Results and discussion

3.1. Antiradical activity screening of the extracts

Four different extracts from cultivated *C. citratus* leaves were assessed by the percentage of DPPH radical reduction compared to the control, in order to screen the most active extract. The results obtained were: infusion, 62.3%; 80% aqueous ethanol, 58.7%; decoction, 48.5% and methanol, 38.6%. Thus the first extract had the strongest activity. The infusion is also the extract most frequently used, either as an aromatic drink, or in traditional medicine. So, a full study was developed from it. To better analyse the phenolic composition and its antioxidant activity contribution, an essential oil-free infusion was used in the subsequent study, which is referred to as infusion.

3.2. Polyphenolic composition and antioxidant activity correlation

HPLC–PDA analyses from *C. citratus* infusion were consistent with the major presence of phenolic acids and their derivatives (peaks 1–4), and of luteolin and apigenin derivatives (peaks 5, 8–14 and peaks 6–7, respectively) (Fig. 2) by comparing their UV spectra with those of standards and/or with those referred to by Mabry et al. (1970).

A fractionation of the phenolic compounds was carried out according to the scheme of Fig. 1. Three fractions: FI (10 mg of dry weight), FII (20.75 mg of dry weight) and FIII (14 mg of dry weight) were obtained from the infusion (230 mg of freeze-dried material), each fraction representing respectively, around 4.3, 9, and 6.1% of the infusion weight. HPLC–PDA analyses suggested that FII contains caffeic acid and caffeic and *p*-coumaric acid derivatives, and FIII contains flavonoids. The HPLC profile of FI is characterized by the occurrence of a hump in the chromatogram and a few compounds showing UV spectrum

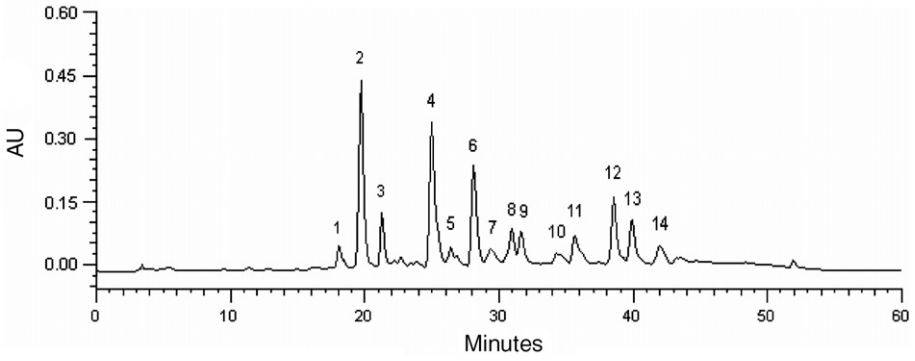


Fig. 2. HPLC–PDA profile of the infusion from *Cymbopogon citratus* leaves recorded at 320 nm. Peaks: 1 and 3, caffeic acid derivatives; 2, caffeic acid; 4, *p*-coumaric acid derivative; 5 and 8 to 14, luteolin derivatives; 6 and 7, apigenin derivatives. For chromatographic conditions see Section 2.

Table 1
Free radical-scavenging activity of the *Cymbopogon citratus* fractions

Fractions	DPPH·	Superoxide anion	Hydroxyl radical
	EC50[a]		
FI (Tannins)	11.0 ± 0.55	4.47 ± 0.22	835 ± 39.8
FII (Phenolic acids)	129 ± 6.64	90.5 ± 4.25	822 ± 40.1
FIII (Flavonoids)	16.9 ± 0.58	13.4 ± 0.76	296 ± 14.8

[a] Amount of the fractions (μg of dry weight) in the reaction mixtures that decreased 50% of the absorbance values as compared to the control assay, i.e. the assay without the fraction under study. Each value is the mean ± SD of three replicates.

similar to flavanols, suggesting the probable presence of condensed tannins (proanthocyanidins) in this fraction. This polyphenolic chemical composition may contribute, at least in part, to some traditional therapeutic properties attributed to this plant that are related to the reactive oxygen species (ROS) scavenging capacity.

The tannin and flavonoid fractions were found to contain the main polyphenolic compounds responsible for the scavenging activity of *C. citratus* infusion, against the ROS assayed: superoxide anion ($O_2^{\cdot-}$) and hydroxyl radical ($HO^{\cdot}$) (Table 1). The ROS react with cellular molecules and contribute to a great variety of diseases. There are great differences in their reactivities; the $HO^{\cdot}$ is highly reactive with almost all biological molecules while the $O_2^{\cdot-}$ is several-fold less reactive. However, as the superoxide anion is the first radical in the oxidative pathway, its neutralization may be an important way to prevent subsequent processes and also the appearance of the hydroxyl radical (Imlay, 2003).

The flavonoid fraction demonstrated a good scavenger capacity for superoxide anion. Additionally, it was the more efficient fraction for hydroxyl radical-scavenging. Consequently, the compounds of this fraction can have a protective effect against those reactive species which are involved in inflammatory and degenerative diseases.

3.3. Structural characterization of the flavonoids

3.3.1. General

The flavonoids were identified essentially on the basis of the HPLC–PDA–ESI/MS^n analysis (Table 2). A combination with TLC and HPLC behaviours before and/or after acid hydrolysis and UV spectral shifts according to Mabry et al. (1970) was also used for the isolated flavonoids.

On-line UV–visible spectra of the flavonoids are typical of flavones according to Mabry et al. (1970). In fact, these compounds exhibited two major absorption bands in the UV region: band I absorption occurring in the 330–351 nm range and band II in the 254–272 nm range (Table 2). However, two different groups of flavones were detected: a group (compounds **1** and from **4** to **13**) with a band II having two absorption maxima (or one maximum with a shoulder) similar to luteolin derivatives, and another group (compounds **2** and **3**) with one absorption maximum corresponding to apigenin derivatives.

3.3.2. Di-C-glycosylflavones

In di-*C*-glycosylflavones (Fig. 3), the main fragments are related to cross-ring cleavages in the sugar units, more extensive fragmentation being for the C-6 sugar residue (Cuyckens & Claeys, 2004; Ferreres, Silva, Andrade, Seabra, & Ferreira, 2003).

Compound **1**. MS analysis showed a pseudomolecular ion at *m/z* 579 and a fragmentation pattern similar to those of asymmetric di-*C*-glycosides. MS^2 data showed fragments at *m/z* 489 $[(M-H)-90]^-$ and 459 $[(M-H)-120]^-$, indicating the presence of a *C*-hexosyl unit. In the same spectrum we could observe the fragments at *m/z* 519 $[(M-H)-60]^-$, 399 $[(M-H)-120-60]^-$ and 369 $[(M-H)-120-90]^-$, corresponding to the fragmentation of a pentosyl unit. A base peak at *m/z* 459 $[(M-H)-120]^-$ and the high abundance of the fragmentation peaks for the hexosyl unit relative to the pentosyl, suggested that a hexose at position 6 occurs in this compound. The low abundance of peak at *m/z* 519 $[(M-H)-60]^-$, which is a result of a characteristic fragmentation of pentoses, corroborated this substitution pattern. The ions at *m/z* 369 (aglycone + 83) and 399 (aglycone + 113), which are characteristic of the di-*C*-glycosylflavone fragmentation (Ferreres et al., 2003), suggested the tetrahydroxylated nature of the aglycone (luteolin, MW 286). Consequently, the compound **1** can be related to a 6-*C*-hexosyl-8-*C*-pentosyl luteolin.

Compound **2** showed a pseudomolecular ion at *m/z* 563 and a fragmentation pattern typical of the asymmetric di-*C*-glycosides. MS^2 data showed fragments at *m/z* 473 $[(M-H)-90]^-$ and 443 $[(M-H)-120]^-$, indicating the presence of a *C*-hexosyl unit. In the same spectrum a fragment was observed at *m/z* 503 $[(M-H)-60]^-$, corresponding to the fragmentation of a pentose. The base peak at *m/z* 473 $[(M-H)-90]^-$ and the high abundance of the fragment at *m/z* 503 $[(M-H)-60]^-$ indicate the presence of a 6-*C*-pentosyl unit. The ions at *m/z* 353 (aglycone + 83) and 383 (aglycone + 113) supported the conclusion that apigenin (MW 270) was the aglycone for compound **2**, whose structure will correspond to 6-*C*-pentosyl-8-*C*-hexosyl apigenin.

Compound **3** presented the same UV spectrum and pseudomolecular ion as compound **2**. A similar fragmentation pattern was also observed. Its general structure could be 6-*C*-pentosyl-8-*C*-hexosyl apigenin.

Compounds **6** and **7** present the same mass (pseudomolecular ions at *m/z* 549) and the same MS^2 spectra fragmentation patterns, which suggested the presence of two pentosyl units linked at positions 6 and 8. On the basis of these results, a 6-*C*-pentosyl-8-*C*-pentosyl luteolin structure was proposed for compounds **6** and **7**.

Compound **10** is a luteolin derivative because it exhibited a characteristic UV spectrum. MS analysis showed a pseudomolecular ion at *m/z* 563 and an MS^2 fragmentation pattern of an asymmetric di-*C*-glycoside. The fragments at *m/z* 519 $[(M-H)-44]^-$, 489 $[(M-H)-74]^-$, 459 $[(M-H)-104]^-$ and 429 $[(M-H)-134]^-$ indicated the presence of a *C*-deoxyhexosyl unit. Fragments at *m/z* 503

Table 2
Flavonoid characterization of the fraction FIII, obtained from a *Cymbopogon citratus* infusion, by HPLC–PDA–ESI/MS^n

Flavonoids		R_t (mm)	λ_{max} by HPLC–PDA (nm)	$[M-H]^-$	MS^2	MS^3
Compound	Partial identification					
1	6-*C*-Hexosyl-8-*C*-pentosyl luteolin	19.30	258sh, 270, 346	579	561(5), 519(9), 489(40), 459(100), 441(12), 429(14), 399(29), 369(22)	441(33), 399(100), 369(77)
2	6-*C*-Pentosyl-8-*C*-hexosyl apigenin	21.15	271, 330	563	545(28), 503(90), 473(100), 443(80), 425(20), 413(17), 383(65), 353(86)	383(9), 353(100)
3	6-*C*-Pentosyl-8-*C*-hexosyl apigenin	21.15	272, 333	563	545(22), 503(68), 473(100), 443(63), 425(18), 413(10), 383(69), 353(65)	383(11), 353(100)
4	6-*C*-Glucosyl luteolin (isoorientin)	22.18	257, 269, 292sh, 351	447	429(29), 357(100), 327(88)	339(100), 297(43), 285(36)
5	2″-*O*-Rhamnosyl isoorientin	22.18	258sh, 269, 291sh, 350	593	503(1), 473(100), 429(71), 369(9), 357(25), 339(8), 309(11), 447(3)	327(100), 298(22)
6	6-*C*-Pentosyl-8-*C*-pentosyl luteolin	23.30	259sh, 270, 292sh, 350	549	531(10), 489(29), 459(100), 441(15), 429(13), 399(37), 369(11)	441(13), 399(100), 369(97)
7	6-*C*-Pentosyl-8-*C*-pentosyl luteolin	25.93	259sh, 270, 292sh, 350	549	531(11), 489(24), 471(11), 459(100), 441(13), 429(17), 399(44), 369(16)	441(33), 399(74), 369(100), 314(28)
8	7-*O*-Glucosyl luteolin	27.10	254, 267sh, 290sh, 345	447	327(3), 285(100)	257(49), 243(59), 241(100), 217(37), 213(53), 199(91), 175(88), 149(87)
9	7-*O*-Neohesperosyl luteolin	27.10	254, 267sh, 348	593	447(100), 327(18), 285(98), 284(23)	327(9), 285(100)
10	6-*C*-Pentosyl-8-*C*-deoxyhexosyl luteolin	28.04	259sh, 270, 292sh, 350	563	545(19), 519 (2), 503(13), 489(14), 473(100), 459(16), 429(11), 399(34), 369(16)	455(13), 399(100), 369(73)
11	6-*C*-Pentosyl luteolin	29.28	257, 270, 291sh, 348	417	399(22), 357(100), 327(35)	339(100), 327(16), 311(20), 297(87), 285(17)
12	*X*″-*O*-Rhamnosyl *C*-pentosyl luteolin	29.28	257, 270, 291sh, 348	563	503(1), 473(100), 417(9), 399(56), 357(24)	327(100), 298(20)
13	*X*″-*O*-Rhamnosyl *C*-(6-deoxy-pento-hexos-ulosyl) luteolin	32.35	260sh, 268, 350	575	531(18), 429(29), 411(100), 367(86), 357(17), 337(13)	367(82), 337(100), 309(10)

sh: shoulder.

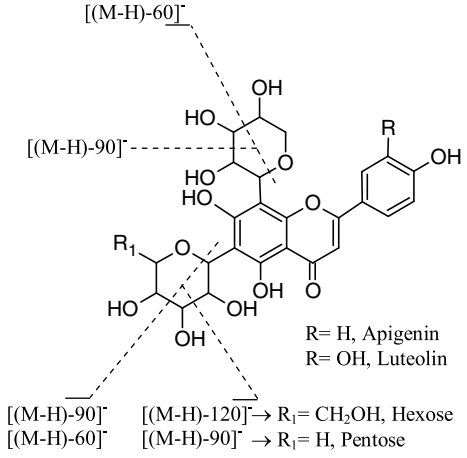


Fig. 3. Fragmentation pattern of the sugar units of di-*C*-glycosylflavones.

$[(M-H)-60]^-$ and 473 $[(M-H)-90]^-$ correspond to a pentose substitution. The base peak $[(M-H)-90]^-$ and the abundance of the fragments related to the pentosyl unit suggested a 6-*C*-pentosylation. Therefore, for compound **10**, the structure of a 6-*C*-pentosyl-8-*C*-deoxyhexosyl luteolin was established.

3.3.3. Mono-C-glycosylflavones

The compounds with this substitution pattern were identified according to the fragmentation proposed by Ferreres et al. (2003).

Compound **4** exhibited a typical luteolin derivative UV spectrum and a pseudomolecular ion at *m/z* 447, that is 132 a.m.u. lower than that of compound **1** (less a pentose unit). The typical fragmentation pattern of a *C*-glycoside, presenting major fragments at *m/z* 357 $[(M-H)-90]^-$ and 327 $[(M-H)-120]^-$, was observed. The presence of the ion at *m/z* 327 $[(M-H)-120]^-$ and the absence of the fragment $[(M-H)-60]^-$ indicated a hexose as the sugar of the *C*-glycosylation. The higher abundance of fragment at *m/z* 357 $[(M-H)-90]^-$ (base peak) suggested that the mono-*C*-glycosylation is in position 6. As glucose is the most representative hexose in nature, compound **4** was identified as 6-*C*-glucosyl luteolin. Comparison with a reference compound suggested the identity of that compound as isoorientin.

Compound **11** has the luteolin UV spectrum and a pseudomolecular ion at *m/z* 417, 30 a.m.u. lower than compound **4**. A pentosyl unit, bound to the aglycone, probably exists. In fact, the MS^2 of compound **11** presented the frag-

ments at *m/z* 357 $[(M-H)-60]^-$ and 327 $[(M-H)-90]^-$, the base peak being the first. These results suggest a 6-*C*-pentosyl luteolin.

3.3.4. *O,C*-Diglycosylflavones

In the *O,C*-diglycosides, the aglycone ion is not detected. Only the precursor $[M-H]^-$ fragments, as well as the ion of the interglycosidic linkage cleavage, occur (Cuyckens & Claeys, 2004). The mass spectra were essentially interpreted on the basis of the fragmentation scheme that is proposed in Fig. 4.

Compound **5** had a pseudomolecular ion at *m/z* 593 that can reveal a luteolin glycoside with a hexose and deoxyhexose. A fragment at *m/z* 447 $[(M-H)-146]^-$, corresponding to loss of one deoxyhexose, with a very low relative intensity (3%) and another at *m/z* 429 $[(M-H)-146-H_2O]^-$ with higher intensity (71%), as well as the absence of the aglycone ion is consistent with an *O,C*-diglycoside structure. According to Cuyckens and Claeys (2004) a relative intensity upper to 90% for the $[(M-H)-146]^-$ ion will be indicative of a di-*O,C*-glycoside. On this basis, compound **5** could have a deoxyhexosyl unit in terminal position of a disaccharide unit. MS^2 data also exhibited fragments at *m/z* 473 $[(M-H)-120]^-$ (base peak) and a minor ion at *m/z* 503 $[(M-H)-90]^-$. That result indicated the presence of a *C*-hexosyl unit.

UV λ_{max} data: (MeOH) 259sh, 270, 351; (+NaOMe) 267, 277sh, 338sh, 392, 409; (+$AlCl_3$) 276, 305sh, 334sh, 428; (+$AlCl_3$+HCl) 265sh, 278, 298sh, 363, 387; (+NaOAc) 272, 278sh, 331sh, 392, 409; (+NaOAc+H_3BO_3) 245sh, 266, 378, 434sh, denoted that the behaviour of compound **5** is typical of a luteolin derivative, presenting a free *ortho*-hydroxylation in the B-ring (3′, 4′) and also hydroxylation at positions 5 and 7. Since all the positions for a direct *O*-link to the aglycone were free, the deoxyhexose unit must be linked to the *C*-hexosyl unit. After acid hydrolysis, two isomers were extracted with *n*-butanol indicating a Wessely–Moser rearrangement (Markham, 1982, chap. 4), as expected for a *C*-glycoside. HPLC analysis of these compounds showed that they had the same retention times

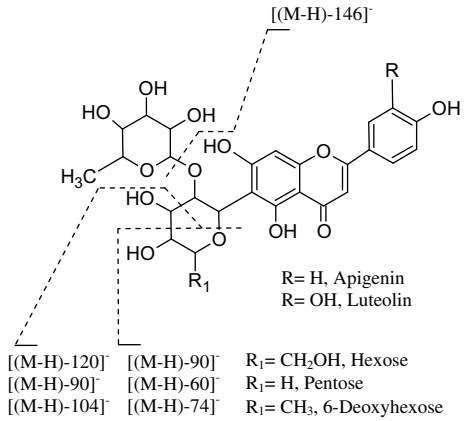


Fig. 4. Fragmentation pattern of the sugar units of *O,C*-diglycosylflavones.

as the standards of the *C*-glucosyl luteolin isomers, isoorientin and orientin, isoorientin being the more representative peak. The sugar analysis by TLC (S1) of the hydrolysed showed the presence of rhamnose, confirming that it corresponded to the *O*-linked deoxyhexose released as a result of the acid hydrolysis.

All in all, compound **5** was identified as *X″*-*O*-rhamnosyl isoorientin, eventually the 2″-*O*-rhamnosyl isoorientin; an assumption supported by the previous identification of the same compound in *C. citratus* extracts (Cheel, Theoduloz, Rodriguez, & Schemeda-Hirschmann, 2005; De Matouschek & Stahl-Biskup, 1991).

Compound **12** showed a pseudomolecular ion at *m/z* 563, 30 a.m.u. lower than that of compound **5**. An MS^2 fragmentation pattern typical of the *O,C*-diglycosylflavones, together with a very weak ion at *m/z* 417 $[(M-H)-146]^-$ (9%), resulting from the loss of a deoxyhexose unit by the cleavage of a interglycosidic linkage, and a major fragment at *m/z* 399 $[(M-H)-146-18]^-$ (56%) were also verified. The absence of fragment $[(M-H)-120]^-$, together with the presence of ions at *m/z* 503 $[(M-H)-60]^-$ and 473 $[(M-H)-90]^-$, revealed that a pentose exists directly linked to the aglycone. This suggested an *O*-deoxyhexosyl *C*-pentosyl luteolin structure.

The UV λ_{max} data: (MeOH) 257, 270, 291sh, 348; (+NaOMe) 268, 276sh, 338sh, 390sh, 408; (+$AlCl_3$) 277, 305sh, 331sh, 427; (+$AlCl_3$+HCl) 262sh, 277, 299sh, 353sh, 389; (+NaOAc) 271, 328sh, 394, 407; (+NaOAc+H_3BO_3) 267, 313sh, 377, 431sh, indicated free hydroxylation at the 5, 7 positions and one B-ring with 3′,4′-dihydroxylation. Acid hydrolysis led to the formation of two isomers, due to a Wessely–Moser rearrangement. TLC analysis for sugars revealed the presence of rhamnose. As all the hydroxyl groups of the luteolin are free, the rhamnose must be *O*-linked to the *C*-glycoside. These results reinforce the conclusion that compound **12** is an *O,C*-diglycoside of luteolin, which corroborates the structure inferred from the mass spectrum. Therefore, the structure proposed corresponds to *X″*-*O*-rhamnosyl *C*-pentosyl luteolin.

Compound **13** presented a pseudomolecular ion at *m/z* 575 and a MS^2 fragmentation pattern typical of an *O,C*-diglycosylflavone; with a low intensity of the fragment at *m/z* 429 $[(M-H)-146]^-$ (29%), a base peak at *m/z* 411 $[(M-H)-146-H_2O]^-$ and the absence of the aglycone ion. These results suggested a deoxyhexosyl unit in the terminal position of a disaccharide unit. The MS^3 spectrum showed two signals, at *m/z* 367 and 337, a result of the loss of 44 and 74 a.m.u., respectively, which is characteristic of the fragmentation of a *C*-linked 6-deoxyhexose. However, an unusual third fragment occurred at *m/z* 309, indicating a loss of 102 a.m.u. (instead of 104 a.m.u.). This result could indicate that this sugar residue has a ketone carbon.

The UV λ_{max} data: (MeOH) 260, 268, 350; (+NaOMe) 267, 280sh, 340sh, 392, 410; (+$AlCl_3$) 276, 304sh, 393sh, 426; (+$AlCl_3$+HCl) 263sh, 278, 298sh, 362sh, 388; (+NaOAc) 271, 279sh, 330sh, 392; (+NaOAc+H_3BO_3) 268, 307sh, 378, 434 indicated hydroxylation at positions 5 and 7 and

3′,4′-dihydroxylation at the B-ring. Acid hydrolysis led to the formation of rhamnose and an acid-isomerization from the Wessely–Moser rearrangement also occurred. These results corroborated an *O*,*C*-diglycosyl luteolin structure, indicated by the interpretation of the mass spectrum. On the basis of all this evidence the structure *X*″-*O*-rhamnosyl *C*-(6-deoxy-pento-hexos-ulosyl) luteolin was established for compound **13**.

3.3.5. *O*-glycosylflavones

Compound **8** presented a pseudomolecular ion at *m*/*z* 447 and the MS^2 revealed the loss of one hexose unit and the appearance of the aglycone fragment at *m*/*z* 285 $[(M-H)-162]^-$ (luteolin), due to the cleavage at the glycosidic *O*-linkage.

UV λ_{max} data: (MeOH) 254, 267sh, 290sh, 345; (+NaOMe) 261, 399; (+$AlCl_3$) 273, 299sh, 330sh, 429; (+$AlCl_3$+HCl) 263sh, 273, 295sh, 358, 386; (+NaOAc) 258, 266sh, 362sh, 394; (+NaOAc+H_3BO_3) 260, 294sh, 373, 442sh, demonstrated a luteolin derivative with substitution at position 7. Acid hydrolysis yielded luteolin, which was identified by HPLC, by comparing it with a reference compound. TLC analysis of sugars (S1) detected glucose as a hydrolysis product. The R_f values of compound **8**: 0.73, 0.00, 0.50, 0.02, 0.14, 0.15 for six different TLC systems (S2 to S7, respectively) and the retention time in HPLC ($R_t = 27.10$) coincided with the reference compound 7-*O*-glucosyl luteolin, which confirmed the identity of compound **8**.

Compound **9** showed a pseudomolecular ion at *m*/*z* 593, 146 a.m.u. higher than that of compound **8**, suggesting that it contained an additional deoxyhexose molecule. Moreover, MS^2 fragments at *m*/*z* 447 $[(M-H)-146]^-$ (base peak) and 285 $[(M-H)-146-162]^-$ were observed, which corresponded to the loss of the deoxyhexosyl unit and of the disaccharide deoxyhexose–hexose, respectively. This fragmentation pattern is characteristic of *O*-diglycosides according to Cuyckens and Claeys (2004). The results obtained also supported the terminal position for the deoxyhexose and a 1→2 interglycosidic linkage for the disaccharide, because of the higher intensity of the fragment $[(M-H)-146]^-$. In the negative ion mode, this ion is more significant than for the compounds having a rutinoside residue (1→6 interglycosidic linkage) (Cuyckens, Rozenberg, Hoffmann, & Claeys, 2001). These results suggested that compound **9** could be the *O*-neohesperosyl luteolin.

The UV λ_{max} data: (MeOH) 254, 267sh, 348; (+NaOMe) 262, 403; (+$AlCl_3$) 274, 297sh, 336sh, 425; (+$AlCl_3$+HCl) 274, 297sh, 359, 386; (+NaOAc) 239, 261, 399; (+NaOAc+H_3BO_3) 260, 374, showed that compound **9** exhibited the same spectral features as compound **8**. It was also a luteolin derivative with substitution in position 7. However, TLC behaviour (R_f – 0.44, 0.00, 0.53, 0.03, 0.27, 0.15 for S2 to S7, respectively) was clearly different for the systems S2 and S6, pointing to compound **9** being similar to 7-*O*-glucosyl luteolin but with one more sugar. Acid hydrolysis of this compound yielded rhamnose in addition to luteolin and glucose. These results reinforced those of MS and demonstrated that compound **9** was 7-*O*-neohesperosyl luteolin**,** a compound previously identified in this plant (De Matouschek & Stahl-Biskup, 1991).

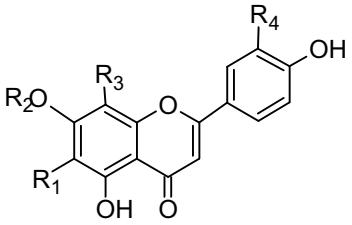
Our extract revealed a high content of flavonoids, representing the flavonoid fraction about 6.1% of the infusion (w/w). Thirteen flavonoids were structurally characterized by HPLC–PDA–ESI/MS^n as glycosylflavones: *O*-glycosides with one or two sugars, *C*-glycosides (mono- and di-) and *O*,*C*-diglycosides (Table 2). Four compounds (isoorientin, 2″-*O*-rhamnosyl isoorientin, 7-*O*-glucosyl luteolin and 7-*O*-neohesperosyl luteolin have already been cited by other investigators (Cheel et al., 2005; De Matouschek & Stahl-Biskup, 1991) and nine were identified for the first time in this study, all of them being *C*-glycosylflavones.

The absorption of flavonoid glycosides is not completely clarified yet. It is supposed that some kind of membrane transporters for these compounds can occur, but the major part of them is hydrolysed during their absorption in the gastrointestinal tract (Walle, 2004). Consequently, systemic effects will be mainly determined by aglycones and/or their conjugated metabolites (Shimoi, Saka, Kaji, Nozawa, & Kinae, 2000), while the *C*-glycosylflavonoids, non-susceptible to acid hydrolysis, could eventually show activity, directly on the gastrointestinal tract.

It has often been reported that glycosylation reduces flavonoid activity, but important properties have been ascribed to glycosylflavonoids, like those identified in this work. Hu and Kitts (2004) documented the inhibition of iNOS and COX-2 for 7-*O*-glucosyl luteolin. Furthermore, Fritsche, Beindorff, Dachtler, Zhang, and Lammers (2002) illustrated a strong inhibitory effect of that flavonoid on 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase activity, resulting in a decreased cholesterol biosynthesis. Some studies also suggest that the glycosyl part probably plays an important role in modulating the effects of flavones (Kwon et al., 2004). Data about the activities of *O*-glycosylflavonoids, namely the 7-*O*-glucosyl luteolin, showed a significant *in vitro* complement inhibiting effect on the classical pathway of the complement system. The complement system might play a role in many diseases with an immune component, such as various forms of arthritis, Alzheimer's disease, asthma, lupus erythematous, autoimmune heart disease and multiple sclerosis (Pieroni, Pachaly, Huang, Van Poel, & Vlietinck, 2000).

Despite the few studies on *C*-glycosylflavonoid biological activities there is evidence that these compounds maintain their antioxidant and antiradical properties associated with very low levels of general toxicity (Kim, Jun, Jeong, & Chung, 2005; Kumarasamy et al., 2004). This work shows that the flavonoid fraction (FIII) contains about 84% of *C*-glycosylflavones (Table 3), and it has significant scavenging activity against the superoxide anion; it is also the most active fraction against the hydroxyl radical (Table 1). Those properties may be indicative of the potential benefit of the flavonoid fraction in the gastrointestinal tract, mainly on ulcerative and erosive lesions generated by free

Table 3
Relative concentration of the flavonoids in the fraction FIII, by HPLC–PDA



R_4= H, apigenin
R_4= OH, luteolin
R_1, R_3= sugar
R_2= H or sugar

Compound	% (Fraction)[a]
6-*C*-Hexosyl-8-*C*-pentosyl luteolin	5.08 ± 0.01
6-*C*-Pentosyl-8-*C*-hexosyl apigenin	3.73 ± 0.01
6-*C*-Pentosyl-8-*C*-hexosyl apigenin	
6-*C*-Glucosyl luteolin (isoorientin)	2.34 ± 0.01
2″-*O*-Rhamnosyl isoorientin	15.1 ± 0.02
6-*C*-Pentosyl-8-*C*-pentosyl luteolin	13.4 ± 0.02
6-*C*-Pentosyl-8-*C*-pentosyl luteolin	6.14 ± 0.01
7-*O*-Glucosyl luteolin	19.5 ± 0.01
7-*O*-Neohesperosyl luteolin	
6-*C*-Pentosyl-8-*C*-deoxyhexosyl luteolin	
6-*C*-Pentosyl luteolin	19.3 ± 0.01
X″-*O*-Rhamnosyl *C*-pentosyl luteolin	
X″-*O*-Rhamnosyl *C*-(6-deoxy-pento-hexos-ulosyl) luteolin	19.1 ± 0.01

[a] Values expressed on the basis of the external standards: isoorientin and vitexin for the luteolin and apigenin derivatives, respectively.

radical activity. Moreover, a fraction containing tannins (FI) was shown to be, of the three fractions, the most active against the superoxide anion. Tannins have been referred to as responsible for most gastrointestinal protective effects. They have radical-scavenger properties and, at low concentrations, are known to create a layer in the mucosa and increase the resistance to chemical and mechanical injury or irritation (Borrelli & Izzo, 2000).

In conclusion, important biological activities are attributed to bioactive polyphenols identified in this work. The *C. citratus* leaves infusion or the flavonoids obtained from it could constitute a potentially important source of compounds with favourable effects for human health.

Acknowledgements

The authors would like to thank the Fundação para a Ciência e Tecnologia (FCT) and Programa Operacional Ciência e Inovação 2010 (POCI)/FEDER for their financial support. The authors are also grateful to Ervital®, Portugal, for the provision of the plant and to J. Paiva for the plant classification.

References

Baratta, M. T., Dorman, H. J. D., Deans, S. G., Figueiredo, A. C., Barroso, J. G., & Ruberto, G. (1998). Antimicrobial and antioxidant properties of some commercial essential oils. *Flavour and Fragrance Journal, 13*(4), 235–244.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature, 181*(4617), 1199–1200.

Borrelli, F., & Izzo, A. A. (2000). The plant kingdom as a source of anti-ulcer remedies. *Phytotherapy Research, 14*(8), 581–591.

Cápiro, N., Sánchez-Lamar, A., Fonseca, G., Baluja, L., & Borges, E. (2001). Capacidad protectora de *Cymbopogon critratus* (DC.) Stapf. ante el dano genético inducido por estrés oxidativo. *Revista Cubana de Investigaciones Biomédicas, 20*(1), 33–38.

Carbajal, D., Casaco, A., Arruzazabala, L., Gonzalez, R., & Tolon, Z. (1989). Pharmacological study of *Cymbopogon citratus* leaves. *Journal of Ethnopharmacology, 25*(1), 103–107.

Cheel, J., Theoduloz, C., Rodriguez, J., & Schemeda-Hirschmann, G. (2005). Free radical scavengers and antioxidants from lemongrass (*Cymbopogon citratus* (DC.) Stapf.). *Journal of Agricultural and Food Chemistry, 53*(7), 2511–2517.

Cuyckens, F., & Claeys, M. (2004). Mass spectrometry in the structural analysis of flavonoids. *Journal of Mass Spectrometry, 39*, 1–15.

Cuyckens, F., Rozenberg, R., Hoffmann, E., & Claeys, M. (2001). Structure characterization of flavonoid *O*-diglycosides by positive and negative nano-electrospray ionization ion trap mass spectrometry. *Journal of Mass Spectrometry, 36*(11), 1203–1210.

De Matouschek, B. V., & Stahl-Biskup, E. (1991). Phytochemische untersuchung der nichtflüchtigen inhaltsstoffe von *Cymbopogon citratus* (DC.) Stapf (Poaceae). *Pharmaceutica Acta Helvetiae, 66*(9–10), 242–245.

Ferreres, F., Silva, B. M., Andrade, P. B., Seabra, R. M., & Ferreira, M. A. (2003). Approach to the study of *C*-glycosylflavones by ion trap HPLC–PAD–ESI/MS/MS: Application to seeds of quince (*Cydonia oblonga*). *Phytochemical Analysis, 14*(6), 352–359.

Figueirinha, A., Cruz, T., Lopes, C., & Batista, T. (2006). Phenolic profile and anti-inflammatory activity of *Cymbopogon citratus* infusion. In *Polyphenols Communications 2006* (pp. 499–500).

Fritsche, J., Beindorff, C. M., Dachtler, M., Zhang, H., & Lammers, J. G. (2002). Isolation, characterization and determination of minor artichoke (*Cynara scolymus* L.) leaf extract compounds. *European Food Research and Technology, 215*(2), 149–157.

Gupta, M. P. (1995). *270 Plantas Medicinales Iberoamericanas*. Colombia: Programa Iberoamericano de Ciencia Y Tecnología para el Desarrollo, CYTED (pp. 308–310).

Hu, C., & Kitts, D. D. (2004). Luteolin and luteolin-7-*O*-glucoside from dandelion flower suppress iNOS and COX in RAW 264.7 cells. *Molecular and Cellular Biochemistry, 265*(1–2), 107–113.

Imlay, J. A. (2003). Pathways of oxidative damage. *Annual Review of Microbiology, 57*, 395–418.

Kasali, A. A., Oyedeji, A. O., & Ashilokun, A. O. (2001). Volatile leaf oil constituents of *Cymbopogon citratus* (DC) Stapf. *Flavour and Fragrance Journal, 16*(5), 377–378.

Kim, Y. C., Jun, M., Jeong, W. S., & Chung, S. K. (2005). Antioxidant properties of flavone *C*-glycosides from *Atractylodes japonica* leaves in human low-density lipoprotein oxidation. *Journal of Food Science, 70*(9), S575–S580.

Kostyuk, V. A., Potapovich, A. I., Vladykovskaya, E. N., & Hiramatsu, M. (2000). Protective effects of green tea catechins against asbestos-induced cell injury. *Planta Medica, 66*(8), 762–764.

Krebs, K. G., Heusser, D., & Wimmer, H. (1969). Spray reagents. In E. Stahl (Ed.), *Thin-layer chromatography. A laboratory handbook* (2nd ed., pp. 857). Berlin: Springer-Verlag.

Kumarasamy, Y., Byres, M., Cox, P. J., Delazar, A., Jaspars, M., Nahar, L., Shoeb, M., & Sarker, S. D. (2004). Isolation, structure elucidation, and biological activity of flavone 6-*C*-glycosides from *Alliaria petiolata*. *Chemistry of Natural Compounds, 40*(2), 122–128.

Kwon, Y. S., Kim, S. S., Sohn, S. J., Kong, P. J., Cheong, I. Y., Kim, C. M., & Chun, W. (2004). Modulation of suppressive activity of lipopolysaccharide-induced nitric oxide production by glycosidation of flavonoids. *Archives of Pharmacal Research, 27*(7), 751–756.

Mabry, T. J., Markham, K. R., & Thomas, M. B. (1970). *The systematic identification of flavonoids, Part II*. Berlin: Springer-Verlag (pp. 35–250).

Markham, K. R. (1982). *Techniques of flavonoid identification*. London: Academic Press (pp. 52–61).

Melo, S. F., Soares, S. F., Costa, R. F., Silva, C. R., Oliveira, M. B. N., Bezerra, R. J. A. C., Caldeira-de-Araújo, A., & Bernardo-Filho, M. (2001). Effect of the *Cymbopogon citratus*, *Maytenus ilicifolia* and *Baccharis genistelloides* extracts against the stannous chloride oxidative damage in *Escherichia coli*. *Mutation Research – Genetic Toxicology and Environmental Mutagenesis, 496*(1–2), 33–38.

Payá, M., Halliwell, B., & Hoult, J. R. S. (1992). Interactions of a series of coumarins with reactive oxygen species: Scavenging of superoxide, hypochlorous acid and hydroxyl radicals. *Biochemical Pharmacology, 44*(2), 205–214.

Pieroni, A., Pachaly, P., Huang, Y., Van Poel, B., & Vlietinck, A. J. J. (2000). Studies on anti-complementary activity of extracts and isolated flavones from *Ligustrum vulgare* and *Phillyrea latifolia* leaves (Oleaceae). *Journal of Ethnopharmacology, 70*(3), 213–217.

Puatanachokchai, R., Kishida, H., Denda, A., Murata, N., Konishi, Y., Vinitketkumnuen, U., & Nakae, D. (2002). Inhibitory effects of lemon grass (*Cymbopogon citratus*, Stapf.) extract on the early phase of hepatocarcinogenesis after initiation with diethylnitrosamine in male Fischer 344 rats. *Cancer Letters, 183*(1), 9–15.

Runnie, I., Salleh, M. N., Mohamed, S., Head, R., & Abeywardena, M. (2004). Vasorelaxation induced by common edible tropical plant extracts in isolated rat aorta and mesenteric vascular bed. *Journal of Ethnopharmacology, 92*(2–3), 311–316.

Shimoi, K., Saka, N., Kaji, K., Nozawa, R., & Kinae, N. (2000). Metabolic fate of luteolin and its functional activity at focal site. *Biofactors, 12*(1–4), 181–186.

Wagner, H., Bladt, S., & Zgainski, E. M. (1984). *Plant drug analysis*. Berlin: Springer-Verlag (p. 303).

Walle, T. (2004). Absorption and metabolism of flavonoids. *Free Radical Biology & Medicine, 36*(7), 829–837.