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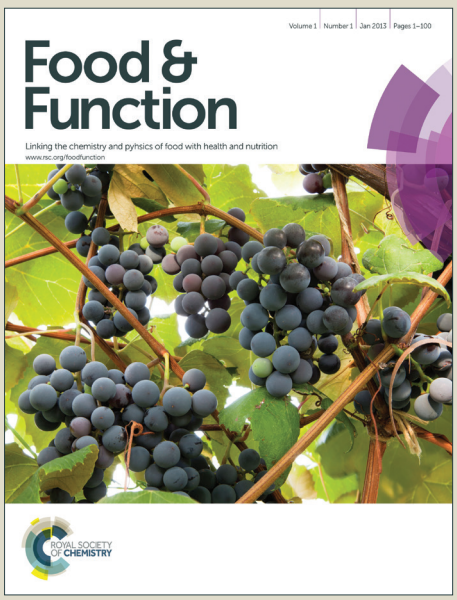

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ARTICLE

Flavan hetero-dimers in *Cymbopogon citratus* infusion tannin fraction and their contribution to the antioxidant activity

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Cymbopogon citratus (lemongrass) leaves infusion, a commonly used ingredient in Asian, African and Latin America cuisines, is also used in traditional medicine for the treatment of several pathologic conditions, however little is known about their bioactive compounds. Recent studies revealed the crucial role of the phenolic compounds on the infusion bioactivity, namely flavonoids and tannins. Flavonoids have already been characterized; however the tannin fraction of lemongrass infusion is still uncharted. The aim of the present work is to characterize this fraction, and to evaluate its contribution to the antioxidant potential of this plant. Chemical characterization was achieved by HPLC-DAD-ESI/tandem MS and antioxidant activity was evaluated using DPPH, ABTS and FRAP assays. Hetero-dimeric flavan structures have been described for the first time in lemongrass consisting of apigeniflavan or luteoliflavan units linked to a flavanone, either naringenin or eriodictyol, which may occur as aglycone or glycosylated forms. The antioxidant capacity of the fraction containing these compounds was significantly higher than the infusion, indicating its potential as a source of natural antioxidants.

Keywords: *Cymbopogon citratus*; lemongrass; Poaceae; polyflavans; polyphenols; tannins; antioxidant.

Introduction

Cymbopogon citratus (DC). Stapf, commonly known as lemongrass, belongs to the Poaceae family and is a tropical perennial shrub originated from Southeast Asia. The lemon-like flavour of the plant is responsible for its use in tropical countries cuisines being its leaves a common ingredient in Asian cuisine in teas, soups and curries, being also suitable for fish, seafood and poultry[1]. In African and Latin American countries, this herb is highly consumed as an aromatic and pleasant-tasting herbal drink. Furthermore, this plant is reported to possess antifungal, mosquito repellent, insecticidal, anti-diabetic, anti-septic, anti-mutagenic and anti-carcinogenic activity. The tea prepared from its leaves is medicinally used in Japan and Brazil[2], and aqueous extracts of dried leaves are used in folk medicine for the treatment of several inflammation-based pathologies[3]. The antioxidant and radical scavenging activities of hydrophilic extracts of *Cymbopogon citratus* have been reported by several authors and related to its polyphenolic components[4–6]. A recent work also revealed the high capacity of an aqueous extract from lemongrass to protect against the hydrogen peroxide-induced oxidative stress[7].

Previous studies suggested that lemongrass contains tannins, namely proanthocyanidins, based on the observation that acid cleavage of a fraction isolated from its lipid-free infusion yielded anthocyanidin-type products, although the precise nature of the compounds was not established[8]. Proanthocyanidins are oligomeric and polymeric flavonoids composed of flavan-3-ol subunits linked by C-C bonds. They are widespread throughout the plant kingdom, where they accumulate in many different organs and tissues, providing protection[9]. Much of the earlier research on proanthocyanidins refers to flavan-3-ol and flavan-3,4-diols oligo/polymers, although other structures able to release anthocyanidins upon heating in acidic alcohol solutions have also been described. Thus, in sorghum, also belonging to the Poaceae family, Gujer and co-workers[10] identified unique hetero-dimers and trimers consisting of a flavanone, either eriodictyol or eriodictyol 5-*O*-glucoside, as the terminal unit linked to one or two glucosylated luteoliflavan (i.e., 5,7,3',4'-tetrahydroxyflavan-5-*O*-glucosyl) extending units. The presence in sorghum of that type of oligomers containing either luteoliflavan or apigeniflavan extending units (i.e., releasing the 3-deoxyanthocyanidins

luteoliflavan and apigeniflavan upon acid cleavage) up to the heptamer was further confirmed by Krueger and colleagues[11].
The present work aims to characterize phytoconstituents of the tannin fraction from *Cymbopogon citratus* infusion, and to evaluate its contribution to the antioxidant potential of this plant.

Results and Discussion

HPLC-DAD-MS analyses

The HPLC-DAD chromatogram of the tannin-rich fraction obtained from an oil-free infusion of *C. citratus* (TF) is shown in Figure 1.

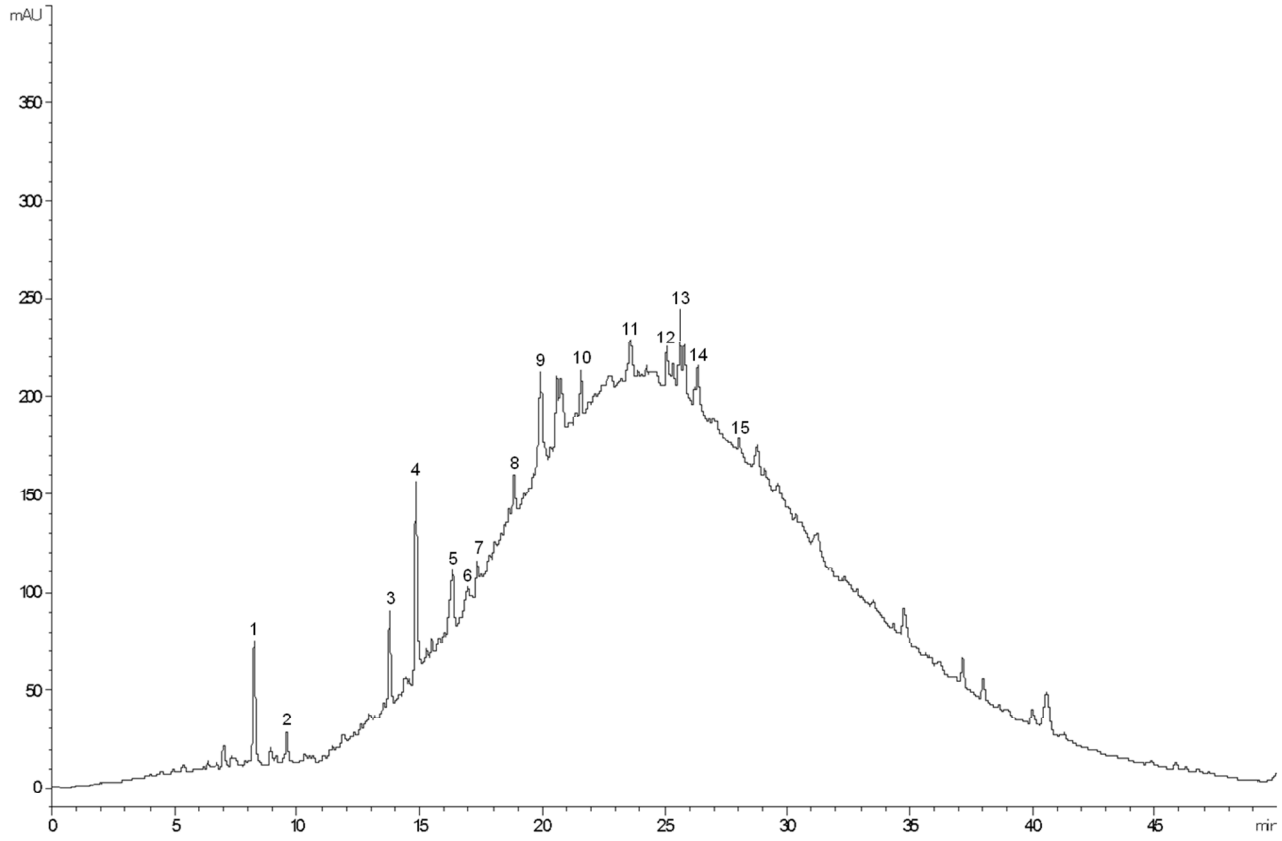


Figure **1**. HPLC-DAD profile of tannin-rich fraction from *Cymbopogon citratus* leaves, registered at 280 nm.

The tentative identities, retention times, UV maxima, and recorded molecular and MS^2 fragment ions for individual components are presented in Table 1.

Table **1**. Phenolic characterization of tannin-rich fraction from *Cymbopogon citratus* infusion, by HPLC-DAD-tandem MS.

Peak	Tentative Identification	Rt (min)	λmaxima (nm)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)
1	Procyanidin B3*	8.25	278	577	451(13), 425(46), 407(100), 289(54)
2	Catechin*	9.59	278	289	245(100), 221(28), 203(50), 163(45), 137(22), 125(66)
3	Procyanidin B4*	13.78	278	577	451(30), 425(65), 407(91), 289(100)
4	Procyanidin B2*	14.85	278	577	451(33), 425(72), 407(94), 289(100)
5	Luteoliflavan-*O*-hexosyl-eriodictyol-*O*-hexoside	16.34	280, 338	883	747(11), 721(100), 559(77)
6	Apigeniflavan-*O*-hexosyl-eriodictyol-*O*-hexoside	17.34	280, 338	867	747(5), 731(2), 705(100), 543(86)

7	Luteoliflavan-*O*-hexosyl-naringenin-*O*-hexoside	18.85	280, 342	867	747(2), 731(3), 705(100), 543(74)
8	Apigeniflavan-*O*-hexosyl-naringenin-*O*-hexoside	19.95	280, 339	851	731(3), 689(100), 527(80)
9	Apigeniflavan-eriodictyol-*O*-hexoside	20.61	280, 331	705	585(16), 569(5), 543(100), 287(2)
10	Luteoliflavan-eriodictyol-*O*-hexoside	21.58	280, 349	721	585(13), 559(100), 287(3)
11	Luteoliflavan-naringenin-*O*-hexoside	23.60	279, 344	705	585(2), 569(4), 543(100), 271(2)
12	Luteoliflavan-naringenin	25.09	280, 342	543	423(31), 407(100), 272(3), 271(15)
13	Apigeniflavan-naringenin-*O*-hexoside	25.71	278, 335sh	689	569(5), 527(100), 271(2)
14	Apigeniflavan-naringenin	26.30	279, 336	527	407(100), 271(11), 256(2)
15	Luteoliflavan-eriodictyol	28.77	278, 341	559	423(100), 287(13), 272(3)

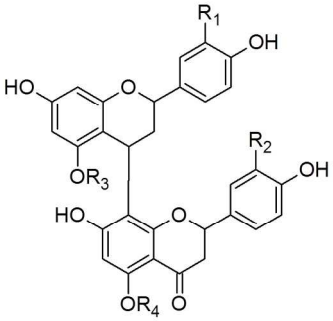
sh: shoulder; *confirmed by a commercial standard

A total of 15 compounds were tentatively identified including two distinct types of flavonoids: flavan-3-ols and flavan-flavanone hetero-dimers, these latter being the most abundant ones.

Compounds 1-4 showed a UV spectral shape characteristic of flavan-3-ols with a single maximum at 278 nm. Compound 2 ($[M-H]^-$ at *m/z* 289) was identified as catechin as confirmed by comparison with a commercial standard. Compounds 1, 3 and 4 were identified as dimeric procyanidins based on their molecular ion ($[M-H]^-$ at *m/z* 577) and MS^2 fragmentation pattern showing the typical *retro* Diels-Alder fission (RDA; -152 amu, *m/z* at 425), RDA + water loss (-170 amu, *m/z* at 407), heterocyclic ring fission (-126 amu, *m/z* at 451), and cleavage of the interflavanic bond following the quinone-methide mechanism (QM; -288 amu, *m/z* at 289). By comparison with the relative retention times of procyanidins previously identified in the laboratory, it was possible to identify compounds 1, 3 and 4 as the procyanidin dimers B3, B4 and B2, respectively.

Compounds 5-15 exhibited a different profile of UV spectrum, showing maximum absorbance at 278-280 nm but also an inflexion between 331 and 349 nm, and their mass spectra suggest that they could correspond to flavan-flavanone hetero-dimers

similar to those reported in sorghum[10,11]. The detected compounds belonged to a series of dimers differing in masses of ±16 amu and ±162 amu depending on the number of hydroxy groups on ring-B and/or hexosyl residues, respectively, and their identities were assigned based on their molecular ions and MS^2 fragmentation patterns. It has been indicated that the position of elementary units in proanthocyanidin oligomers could be deduced through the analysis of the product ions derived from the quinone methide (QM) cleavage of the interflavan bond, where lower (terminal) units would be released as such, while the upper (extension) units suffer a structural rearrangement yielding ions 2 Da lower than the original flavan constituents[12,13]. Thus, for instance, terminal eriodyctiol (MW 288) and narigenin (MW 272) would be expected to produce negative ions at *m/z* 287 and 271, respectively, whilst extension luteoliflavan and apigeniflavan units could be expected to produce them at *m/z* 272 and 256, respectively. Additional support to deduce the position of the units could be obtained from the fragments corresponding to the retro-Diels-Alder (RDA) cleavage that has been reported to occur mainly in the upper subunit of the proanthocyanidins[12,13]. Tentative identities for compounds 5-15 were assigned based on those assumptions and are indicated in Table 1 and their structures depicted in Figure 2.



Compound	R_1	R_2	R_3	R_4
5	OH	OH	hex	hex
6	H	OH	hex	hex
7	OH	H	hex	hex
8	H	H	hex	hex
9	H	OH	H	hex
10	OH	OH	H	hex
11	OH	H	H	hex
12	OH	H	H	H
13	H	H	H	hex
14	H	H	H	H
15	OH	OH	H	H

hex: hexosyl residue

Figure **2**. Proposed structures for compounds **5**-**15** identified in tannin-rich fraction from *Cymbopogon citratus* leaves.

Compound 12 presented a molecular ion $[M-H]^-$ at *m/z* 543 releasing MS^2 fragment ions at *m/z* 423 (-120 amu) and 407 (-136 amu) from the RDA fission of mono- and di-hydroxylated B-ring units, respectively; the higher abundance of the ion at *m/z* 407 suggested that it was produced from the cleavage of the upper (extension) unit of a di-hydroxylated B-ring, which was, therefore, associated to a luteoliflavan. The other two product ions at *m/z* 272 and 271 would derive from the interflavan cleavage and could be attributed to the upper luteoliflavan unit and a terminal naringenin, respectively. Thus, the compound was tentatively identified as the dimer luteoliflavan-naringenin. Similar reasoning was applied to assign compounds 14 and 15 as the dimers apigeniflavan-naringenin and luteoliflavan-eriodictyol, respectively.

The other hetero-dimers contained one or two hexosyl substituents linked to the aglycone(s) through an oxygen bridge, as deduced from the observation of losses of 162 amu in their MS^2 fragmentation spectra. Compound 9 ($[M-H]^-$ at *m/z* 705) showed a majority product ion at *m/z* 543 (-162 amu, loss of a hexosyl residue); the ion at *m/z* 287 would be due to further QM cleavage of the previous ion, suggesting that eriodictyol hexoside would be the terminal unit. The other two fragment at *m/z* 585 (-120 amu) and 569 (-136 amu) could be attributed to RDA fissions of the upper and lower units, respectively, taking into account relative abundances, which suggested an apigeniflavan nature for the extension unit. Therefore, the compound was tentatively assigned as apigeniflavan-

eriodictyol-*O*-hexoside. Based on similar considerations, compounds 10, 11 and 13 were respectively identified as luteoliflavan-eriodictyol-*O*-hexoside, luteoliflavan-naringenin-*O*-hexoside and apigeniflavan-naringenin-*O*-hexoside.

Compound 8 ($[M-H]^-$ at *m/z* 851) showed major fragment ions at *m/z* 689 and 527 from the consecutive loss of two hexosyl residues, whereas another ion at *m/z* 731 (-120 amu) pointed to the existence of an upper apigeniflavan unit, so it could be tentatively identified as apigeniflavan-*O*-hexosyl-naringenin-*O*-hexoside. Similarly, according to its molecular ion, compound 5 was assigned as luteoliflavan-*O*-hexosyl-eriodictyol-*O*-hexoside.

Compounds 6 and 7 presented the same molecular ion at *m/z* 867 and similar fragmentation pattern with two main fragments ions at *m/z* 705 and 543 from the consecutive loss of two hexosyl residues, and other two at *m/z* 747 (-120 amu) and 731 (-136 amu) attributable to RDA cleavages. These compounds were tentatively assigned based on the relative abundances of these latter ions. Thus, the ion at *m/z* 747 was more abundant in compound 6 which might suggest an upper apigeniflavan unit, so that it could be identified as apigeniflavan-*O*-hexosyl-eriodictyol-*O*-hexoside. In contrast the ion at *m/z* 731 was more abundant in compound 7 pointing to an upper luteoliflavan unit, allowing its tentative identification as luteoliflavan-*O*-hexosyl-naringenin-*O*-hexoside. The nature and substitution position of the hexosyl residues on the different aglycones cannot be established from the available data, although they might be speculated to be glucose probably linked to the hydroxyl group at C_5 of the corresponding flavan unit, according to the previous identifications made by Gujer[10] and Krueger[11] in sorghum.

Acid-catalyzed hydrolysis

The product from TF acid hydrolysis was characterized by HPLC-DAD-MS and apigenidin and luteolinidin molecules were detected (data not shown). These results confirm the presence of apigeniflavan and luteoliflavan residues in the structure of the tannin oligomers present in TF, respectively.

Antioxidant activity

Table 2 shows the TEAC values for the lipid-free infusion (CcI) and tannin fraction (TF) of *C. citratus* obtained by different assays.

Table **2**. Antioxidant activity of CcI and TF from *Cymbopogon citratus*.

Sample	TEAC*			
	DPPH•	ABTS• (pH=4)	ABTS• (pH=7)	Fe^{3+}
CcI	1.40±0.06	2.29±0.02	0.83±0.04	2.63±0.10
TF	0.61±0.07	1.59±0.05	0.65±0.06	0.38±0.07

*TEAC (Trolox-Equivalent Antioxidant Capacity): Amount of the samples (mg/mL) that has the same anti-radical activity of Trolox 1 mM. The results are expressed as mean±SD of three independent experiments.

In all assays, TF exhibited higher antioxidant capacity (lower TEAC values). In the DPPH assay, the TEAC value for TF was 0.61, 2.33 times more potent than CcI. Nevertheless, the TEAC value of CcI, which corresponds to an EC_{50} of 28.99μg/mL, is substantially better than another lemongrass infusion previously mentioned in the literature, which presents an EC_{50} of 41.72μg/mL[14]. In the ABTS (pH=4) method, TF and CcI showed TEAC values of 1.59 and 2.29, respectively; whereas in the ABTS (pH=7) assay, the samples gave TEAC values of 0.65 and 0.83, respectively. In both cases, TF was about 1.3 times more efficient in neutralizing the ABTS• radical than CcI. However, the two samples happened to be significantly more potent at pH=7 than in an acidic environment, which might be important when considering physiological conditions. In the FRAP assay, TF (TEAC value of 0.38) was almost 7 times more efficient than CcI (2.63). In the case of CcI, the antioxidant capacity increases as follows: Fe^{3+} < ABTS (pH=4) < DPPH < ABTS (pH=7), while TF antioxidant power increases: ABTS (pH=4) < ABTS (pH=7) < DPPH < Fe^{3+}. This may be explained by the fractionation process, leading to a different matrix and composition with a distinct chemical behaviour against the tested oxidant species. Nevertheless, the tannin fraction proved to be fairly more active in inactivating the potentially harmful oxidant entities than the crude extract obtained by infusion. This data seems to be supported by previous studies in *Sorghum bicolor*, which showed that this type of condensed tannins has a very important contribution to the antioxidant activity of the whole plant[15,16].

Experimental Section

Plant material, extract preparation and fractioning

Dry leaves of *Cymbopogon citratus* were purchased from ERVITAL (Mezio, Castro Daire, Portugal). The plant was cultivated in the region of Mezio, Castro Daire (Portugal). A voucher specimen was deposited in the Herbarium of Aromatic and Medicinal Plants of the Faculty of Pharmacy – University of Coimbra (A. Figueirinha 0109). The identity of the plant was confirmed by J. Paiva (Life Sciences Department, University of Coimbra, Portugal). A lipid- and essential oil-free infusion was prepared (CcI) as previously described [17]. Afterwards, the extract was fractionated on a reverse phase preparative Flash Chromatography® C18 column Buchi® (150 × 40 mm; particle size 40 - 63 μm) (Flawil, Switzerland), eluted with aqueous methanol, in a discontinuous gradient: 5% (0-40 min), 5-10% (40-55 min), 10% (55-85 min), 10-15% (85-90 min), 15-25% (90-110 min), 25-50% (110-140 min), 50% (140-160 min), 50-80% (160-180 min), 80-100% (180-200 min) and 100% (200-220 min) at a flow rate of 3 mL/min. The chromatographic profile was registered at 280 and 320 nm by the UV detector C-640 Buchi® (Flawil, Switzerland), and the data was acquired using the software ECOMAC® 0.238 (Prague, Czech Republic). Two fractions were obtained: F1 (0-120 min), containing phenolic acids and flavonoids and F2 (120-220 min), with flavonoids and tannins. F2 was then sub-fractionated by gel chromatography on a Sephadex® LH-20 (Sigma-Aldrich, Amersham, Sweden) column (35 x 4 cm) using ethanol as mobile phase. All fractionation processes were monitored by high-performance liquid chromatography (HPLC) and thin layer chromatography (TLC) for polyphenols, providing two major fractions: flavonoids fraction, FF (yield 31.5%) and tannins fraction, TF (yield 14.2%). TF was analyzed by HPLC-DAD-ESI/tandem MS.

HPLC-DAD-MS

Analyses were carried out in a Hewlett-Packard 1100 chromatograph (Agilent® Technologies, Waldbronn, Germany) with a quaternary pump and a diode array detector (DAD) coupled to an HP Chem Station (rev. A.05.04) data-processing station. An Agilent Poroshell 120 EC-C18, 2.7 μm (4.6 x 150

mm) column thermostatted at 35 °C was used. The solvents used were: (A) 0.1% formic acid, and (B) acetonitrile. The elution gradient established was from 8% B to 10% B in 5 min, to 25% B in 20 min, to 40% B in 20 min, to 60% B in 5 min and steady at 60% B for more 5 min and re-equilibration of the column using a flow rate of 0.5 mL/min. Double online detection was carried out in the DAD at 280, 320 and 370 nm as preferred wavelengths and in a mass spectrometer (MS) connected to HPLC system via the DAD cell outlet.

MS detection was performed in an API 3200 Qtrap (Applied Biosystems, Darmstadt, Germany) equipped with an ESI source and a triple quadrupole-ion trap mass analyzer, which was controlled by the Analyst 5.1 software. Zero grade air served as the nebulizer gas (30 psi) and as turbo gas (400 ºC) for solvent drying (40 psi). Nitrogen served as the curtain (20 psi) and collision gas (medium). Both quadrupols were set at unit resolution. The ion spray voltage was operated at -4500 V in the negative mode. Method settings were: declustering potential (DP), -40 V; entrance potential (EP), -10 V; collision energy (CE), -50 V; and cell exit potential (CXP) -3 V. In order to obtain the fragmentation pattern of the parent ion, enhanced product ion (EPI) mode was also applied using the following settings: declustering potential (DP), -50 V; entrance potential (EP), -6 V; collision energy (CE), -25 V; and collision energy spread (CES) 0 V.

Acid-catalyzed hydrolysis

In order to confirm the nature of the compounds from TF, an acid-catalyzed hydrolysis was performed according to Porter *et al.*[18] Briefly, an aliquot of TF dissolved in methanol was mixed with sulphuric acid in buthanol (5%, v/v) and iron-amonium sulfate dodecahydrate in hydrochloric acid 2N (2%, w/v). The mixture was sealed inside a glass vial and heated at 95ºC for 40 min. Then, the product of the hydrolysis was microfiltered and analysed by HPLC-DAD-MS.

Antioxidant activity

For the antioxidant activity assays, the adequate amount of freeze-dried sample was solubilised in the referred solvent for each test.

DPPH radical assay

Free radical-scavenging activity was evaluated according to the method described by Blois et al. (1958). Aliquots of samples (100 µL) were assessed by their reactivity with a methanolic solution of 500 µM DPPH (500 µL) (Sigma-Aldrich, Quimica S.A., Portugal) in the presence of 100 mM acetate buffer, pH 6.0 (1 mL). 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. Different dilutions of each of the test compounds were assayed and the results were obtained by interpolating the absorbance on a calibration curve obtained with Trolox (62.5-1000 µM) (Sigma-Aldrich, Quimica S.A., Portugal). Two independent experiments in triplicate were performed for each of the assayed compounds. Results were expressed as Trolox equivalent antioxidant capacity (TEAC), defined as the concentration of the sample solution whose antioxidant capacity is equivalent to a 1.0 mM solution of Trolox[19].

ABTS (pH=4) radical assay

The assay was carried out according to Cano [20] with minor modifications made by Villaño[21]. Free radicals were generated by an enzymatic system consisting of horseradish peroxidase enzyme, its oxidant substrate (hydrogen peroxide) and the 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$) chromophore. The radical was generated by a reaction between 1.5 mM ABTS (Sigma-Aldrich, Spain), 15 µM hydrogen peroxide (Sigma-Aldrich, Spain) and 0.25 µM peroxidase (Sigma-Aldrich, Spain) in 50 mM glycine-HCl buffer (pH 4.5). The final volume was 60 mL, yielding a final concentration of 30 µM of the $ABTS^{\bullet+}$ radical cation. The blank reference cuvette contained glycine–HCl buffer. Once the radical was formed, the sample was added and the decrease in absorbance was monitored. The assay was carried out at room temperature. The reaction started by adding 100 µL of test sample to 2 mL of $ABTS^{\bullet+}$ solution, the samples were vortexed for 10 s, and the absorbance at 414 nm was measured after 2 min of reaction. Two independent experiments in triplicate were performed for each of the assayed compounds. In each case, six different dilutions were prepared in 50% aqueous methanol and submitted to the reaction. TEAC values were obtained by interpolating the decrease in absorbance on the calibration curve obtained using Trolox solutions from 62.5 to 500 µM.

ABTS (pH=7) radical assay

In this assay, the $ABTS^{\bullet+}$ radical was produced by the oxidation of 7 mM ABTS with potassium persulphate (2.45 mM, final concentration) (Merck, Darmstadt, Germany) in water. The mixture was allowed to stand in the dark at room temperature for 12–16 h before use, and then the $ABTS^{\bullet+}$ solution was diluted with phosphate buffered saline (PBS) at pH 7.4 and equilibrated at 30 ºC to give an absorbance of 0.7±0.02 at 734 nm. Aliquots (50 µL) of 50% aqueous methanol of the test compound were mixed with 2 mL of the $ABTS^{\bullet+}$ preparation, vortexed for 10 s, and the absorbance measured at 734 nm after 4 min of reaction at 30 ºC. Different dilutions of each of the test compounds were assayed and the results were obtained by interpolating the absorbance on a calibration curve obtained with Trolox (62.5–500 µM). The results were expressed as TEAC values. Two independent experiments in triplicate were performed for each of the assayed compounds.

Ferric reducing power assay

Ferric reducing ability was evaluated according to Benzie and Strain[22] with minor modifications. The FRAP reagent contained 10 mM of TPTZ (Sigma-Aldrich, Spain) solution in 40 mM HCl, 20 mM $FeCl_3 \cdot 6H_2O$ (Merck, Darmstadt, Germany) and acetate buffer (300 mM, pH 3.6) (1:1:10, v/v/v). Aliquots (100 µL) of 50% aqueous methanol of the test compounds were added to 3 mL of the FRAP reagent, and the absorbance was measured at 593 nm after incubation at room temperature for 6 min, using the FRAP reagent as blank. Different dilutions of each of the test compounds were assayed and the results were obtained by interpolating the absorbance on a calibration curve obtained with Trolox (31.25-1000 µM). The results were expressed as TEAC values. Two independent experiments in triplicate were performed for each of the assayed compounds.

Conclusions

The present work allowed the identification of hetero-dimeric flavan structures for the first time in *Cymbopogon citratus*. These flavonoid oligomers consist of apigeniflavan or luteoliflavan units linked to a flavanone, either naringenin or eriodictyol, and occur as aglycone and glycosylated forms. The detected proanthocyanidin hetero-dimers, along with some

common procyanidin dimers, constitute the main compounds in the tannin fraction of *C. citratus* infusion. The findings of this study support the antioxidant potential of this plant and emphasize the contribution of the tannin fraction to this activity.

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Notes and references

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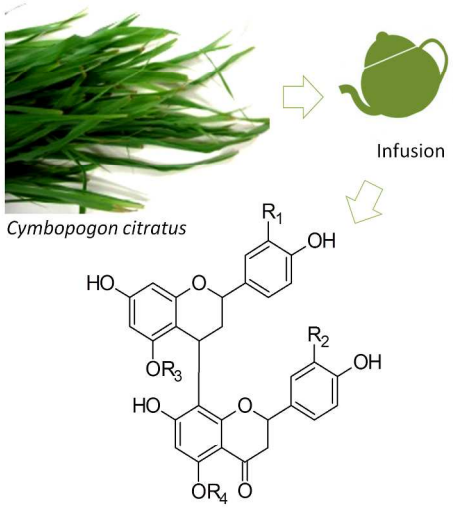
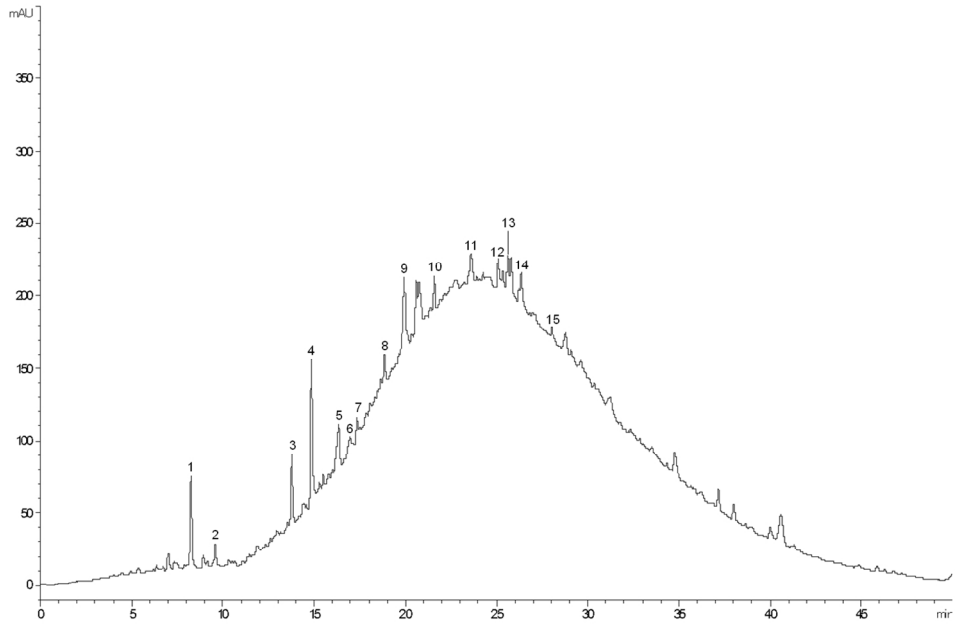
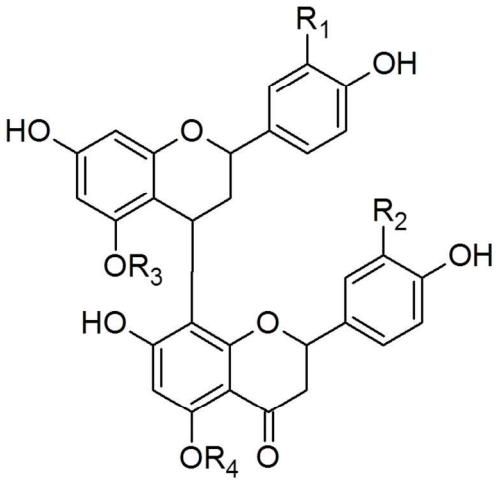


For the first time, flavan hetero-dimers were identified in *Cymbopogon citratus* infusion, by HPLC-DAD-MS These compounds exhibited a pronounced antioxidant capacity and seem to be the main contributors to the overall antioxidant properties of the lemongrass infusion.

848x473mm (150 x 150 DPI)



400x266mm (96 x 96 DPI)



Compound	R_1	R_2	R_3	R_4
5	OH	OH	hex	hex
6	H	OH	hex	hex
7	OH	H	hex	hex
8	H	H	hex	hex
9	H	OH	H	hex
10	OH	OH	H	hex
11	OH	H	H	hex
12	OH	H	H	H
13	H	H	H	hex
14	H	H	H	H
15	OH	OH	H	H

hex: hexosyl residue

355x189mm (96 x 96 DPI)

Table **1**. Phenolic characterization of tannin-rich fraction from *Cymbopogon citratus* infusion, by HPLC-DAD-tandem MS.

Peak	Tentative Identification	Rt (min)	λmaxima (nm)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)
1	Procyanidin B3*	8.25	278	577	451(13), 425(46), 407(100), 289(54)
2	Catechin*	9.59	278	289	245(100), 221(28), 203(50), 163(45), 137(22), 125(66)
3	Procyanidin B4*	13.78	278	577	451(30), 425(65), 407(91), 289(100)
4	Procyanidin B2*	14.85	278	577	451(33), 425(72), 407(94), 289(100)
5	Luteoliflavan-*O*-hexosyl-eriodictyol-*O*-hexoside	16.34	280, 338	883	747(11), 721(100), 559(77)
6	Apigeniflavan-*O*-hexosyl-eriodictyol-*O*-hexoside	17.34	280, 338	867	747(5), 731(2), 705(100), 543(86)
7	Luteoliflavan-*O*-hexosyl-naringenin-*O*-hexoside	18.85	280, 342	867	747(2), 731(3), 705(100), 543(74)
8	Apigeniflavan-*O*-hexosyl-naringenin-*O*-hexoside	19.95	280, 339	851	731(3), 689(100), 527(80)
9	Apigeniflavan-eriodictyol-*O*-hexoside	20.61	280, 331	705	585(16), 569(5), 543(100), 287(2)
10	Luteoliflavan-eriodictyol-*O*-hexoside	21.58	280, 349	721	585(13), 559(100), 287(3)
11	Luteoliflavan-naringenin-*O*-hexoside	23.60	279, 344	705	585(2), 569(4), 543(100), 271(2)
12	Luteoliflavan-naringenin	25.09	280, 342	543	423(31), 407(100), 272(3), 271(15)
13	Apigeniflavan-naringenin-*O*-hexoside	25.71	278, 335sh	689	569(5), 527(100), 271(2)
14	Apigeniflavan-naringenin	26.30	279, 336	527	407(100), 271(11), 256(2)
15	Luteoliflavan-eriodictyol	28.77	278, 341	559	423(100), 287(13), 272(3)

sh: shoulder; *confirmed by a commercial standard

Table **2**. Antioxidant activity of CcI and TF from *Cymbopogon citratus*.

Sample	TEAC*			
	DPPH•	ABTS• (pH=4)	ABTS• (pH=7)	Fe^{3+}
CcI	1.40±0.06	2.29±0.02	0.83±0.04	2.63±0.10
TF	0.61±0.07	1.59±0.05	0.65±0.06	0.38±0.07

*TEAC (Trolox-Equivalent Antioxidant Capacity): Amount of the samples (mg/mL) that has the same anti-radical activity of Trolox 1 mM. The results are expressed as mean±SD of three independent experiments.