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Free Radical Scavengers and Antioxidants from Lemongrass (*Cymbopogon citratus* (DC.) Stapf.)

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Methanol, MeOH/water extracts, infusion, and decoction of *Cymbopogon citratus* were assessed for free radical scavenging effects measured by the bleaching of the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical, scavenging of the superoxide anion, and inhibition of the enzyme xanthine oxidase (XO) and lipid peroxidation in human erythrocytes. The extracts presented effect in the DPPH and superoxide anion assay, with values ranging between 40 and 68% and 15−32% at 33 and 50 μg/mL, respectively, inhibited lipid peroxidation in erythrocytes by 19−71% at 500 μg/mL and were inactive toward the XO at 50 μg/mL. Isoorientin, isoscoparin, swertiajaponin, isoorientin 2″-*O*-rhamnoside, orientin, chlorogenic acid, and caffeic acid were isolated and identified by spectroscopic methods. Isoorientin and orientin presented similar activities toward the DPPH (IC_{50}: 9−10 μM) and inhibited lipid peroxidation by 70% at 100 μg/mL. Caffeic and chlorogenic acid were active superoxide anion scavengers with IC_{50} values of 68.8 and 54.2 μM, respectively, and a strong effect toward DPPH. Caffeic acid inhibited lipid peroxidation by 85% at 100 μg/mL.

KEYWORDS: ***Cymbopogon citratus*; free radical scavengers; antioxidants; flavonoid *C*-glycosides**

INTRODUCTION

Cymbopogon citratus (DC.) Stapf. (*Gramineae*), also known as lemongrass, is a plant cultured in almost all tropical and subtropical countries as a source of essential oil. Lemongrass is used in Peru for preparing soft drinks and is used as an aromatic, pleasant-tasting herbal tea all around its distribution area. The infusion or decoction of its aerial parts has widespread use in folk medicine. The plant is recommended to treat digestive disorders, inflammation, diabetes, nervous disorders, and fever as well as other health problems (*1*).

In the past decade, it has been shown that antioxidants and free radical scavengers are relevant in the prevention of pathologies such as atherosclerosis, heart diseases, cancer, and arthritis, in which reactive oxygen species or free radicals are implicated (*2*). Flavonoids and phenolics from plants have been reported as potent free radical scavengers and frequently occur in medicinal and aromatic plants. Several diseases of the gastrointestinal tract seem to be induced by oxidative stress. The role of oxygen-derived free radicals has been studied in acute gastric and esophageal mucosal injury caused by isquemia, antiinflammatory drugs, or ethanol (*3*, *4*). Administration of free radical scavengers has been found to prevent esophageal mucosal damages (*3*, *4*). Other studies have provided direct evidence of the implication of the oxidative stress in inflammatory diseases of the gastrointestinal tract (*3*, *4*). There is evidence suggesting that oxidative damage plays a prominent role in hepatic injury mediated by drugs and poison (*5*).

Preliminary assays carried out in our laboratory showed antioxidant and free radical scavenging effects of a methanolic extract of *C. citratus*. Since the effect of nutritional antioxidants may be associated with some of the reputed beneficial effects of the crude drug, a study was undertaken to isolate, identify, and quantify the main free radical scavengers and antioxidants from *C. citratus*. This study investigated the activity of different crude extracts and compounds obtained from *C. citratus* aerial parts, assessed by the decoloration of a methanolic solution of DPPH, inhibition of lipid peroxidation in erythrocytes, superoxide anion scavenging effect, and inhibition of xanthine oxidase and cytotoxicity toward human lung fibroblasts.

MATERIALS AND METHODS

Chemicals. All solvents used were of analytical grade. Chloroform, ethanol, and methanol were obtained from J. T. Baker (Phillipsburg, NJ). HPLC-grade acetonitrile and formic acid from Merck (Darmstadt, Germany) were used. The standards of caffeic acid and quercetin were purchased from Sigma Chemical Co. (St. Louis, MO). TLC analysis was carried out on aluminum-coated silica gel (Sigma-Aldrich, St. Louis, MO) and Cellulose F_{254} plates from Merck. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), nitrobluetetrazolium (NBT), thiobarbituric acid, xanthine oxidase, xanthine, hypoxanthine, allopurinol, and neutral red were purchased from Sigma Chemical Co. Culture media, antibiotics, and fetal bovine serum were obtained from Invitrogen Corp. (Carlsbad, CA).

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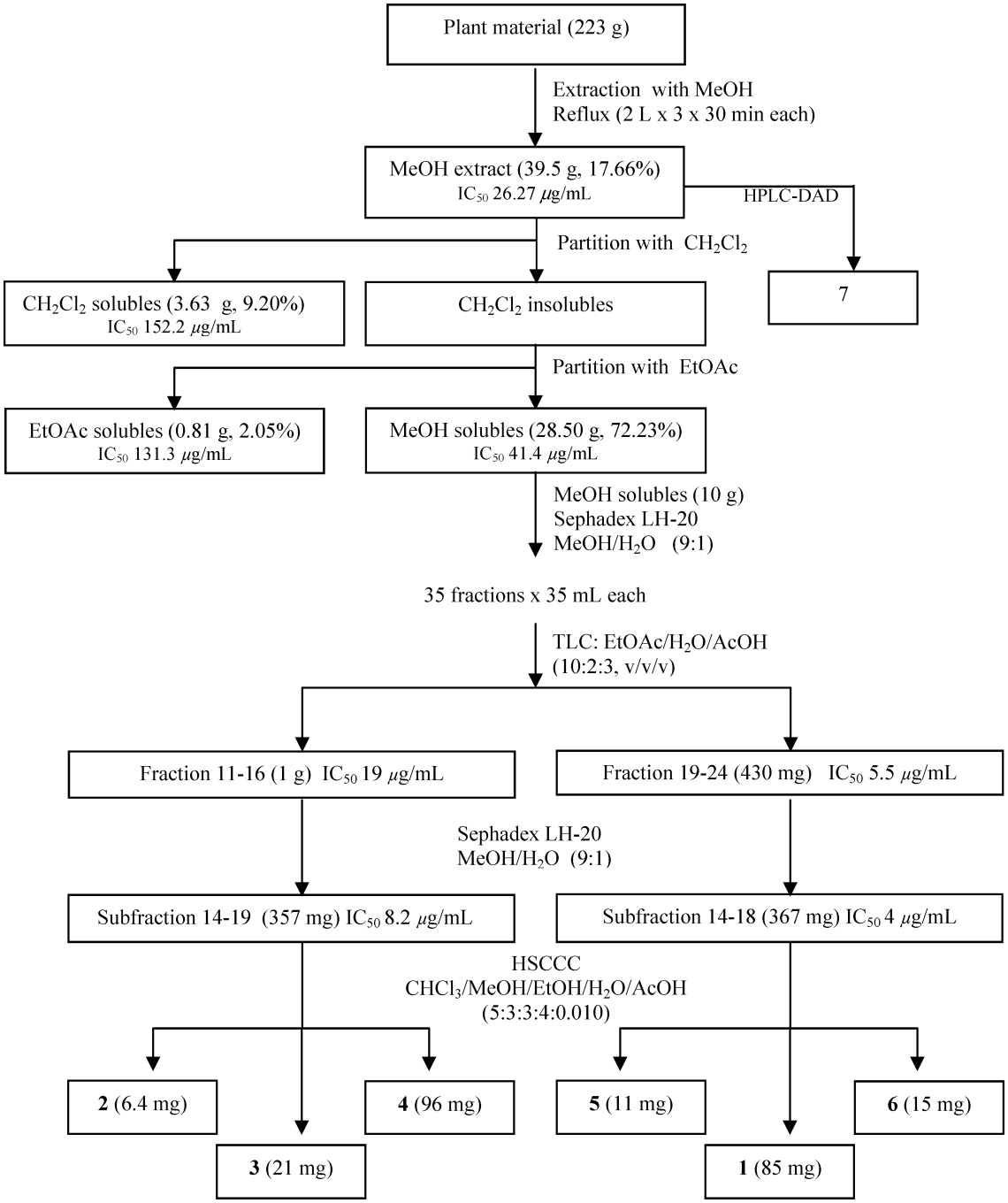


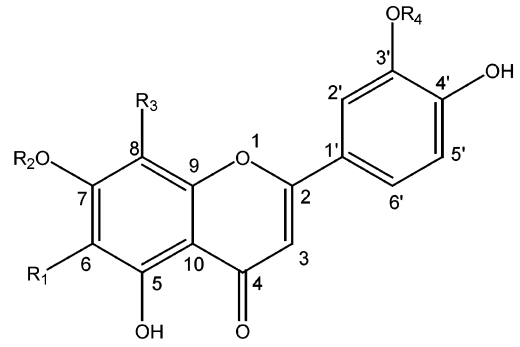
Figure 1. DPPH assay-guided isolation of the free radical scavengers and antioxidants from *C. citratus*.

Plant Material and Extraction. The aerial parts of *C. citratus* were collected from the Botanical Garden of the Universidad de Talca, Chile in February 2003. A voucher specimen (no. 2771) has been kept at the Herbarium of the institution. The air-dried, powdered plant material was extracted as follows. Five representative samples of *C. citratus* (10 g each, in triplicate) were extracted under reflux using methanol/water mixtures (7:3 and 1:1), MeOH, and also hot water (decoction and infusion). The infusion was prepared with 10 g of air-dried, powdered plant material soaked in 250 mL of boiling water for 15 min, and the decoction was performed with 10 g of air-dried, powdered material boiled in 250 mL of water for 2.5 h. The antioxidant profile of the extracts was determined by means of the selected assays.

Extraction and Isolation of the Active Compounds. The MeOH crude extract was the most active and was selected for the isolation and characterization of the main free radical scavengers and antioxidants from lemongrass. Each fraction was assessed for the free radical scavenging effect by the DPPH decoloration test. The isolation process is summarized in **Figure 1**.

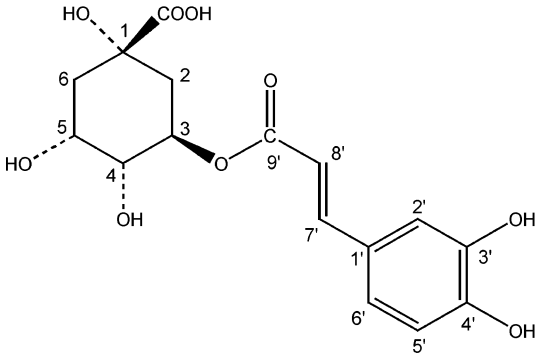
High-Speed Preparative Countercurrent Chromatography (HSCCC). The active fractions 14-19 and 14-18 (see **Figure 1**) were submitted to high-speed preparative countercurrent chromatography (HSCCC) using a P. C. Inc. (Potomac, MD) instrument equipped with a multilayer coil of 1.68 mm i.d. poly(tetrafluoroethylene) (PTFE) tubing of approximately 80 and 240 mL with a total capacity of 320 mL.

The revolution radius or the distance between the holder axis and central axis of the centrifuge (R) was 10.5 cm, and the β value was 0.76 ($\beta = r/R$, where r is the distance from the coil to the holder shaft). The speed varied between 0 → 1200 rpm. The flow rate was controlled with a DC Analytic Gearmotor (Bodine Electric Company, Chicago, IL). The sample was injected with a P. C. Inc. injection module with a 10 mL sample injection loop. The solvent system used was $CHCl_3$/MeOH/EtOH/H_2O/AcOH (5:3:3:4:0.010, v/v/v/v). It was thoroughly equilibrated overnight in a separatory funnel at room temperature, and the two phases were separated and degassed by sonication shortly before use. The solvent system provided an ideal range of the partition

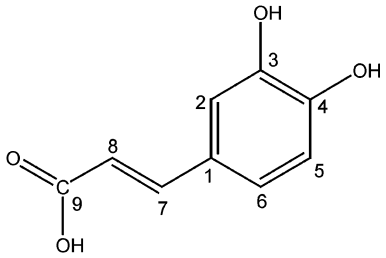


	R_1	R_2	R_3	R_4
1	Glu	H	H	H
2	Glu	H	H	CH_3
3	Glu	CH_3	H	H
4	Glu-Rh	H	H	H
5	H	H	Glu	H

Compounds **1-5**



Compound **6**



Compound **7**

Figure 2. Compounds isolated from the polar extract of *C. citratus*.

coefficient (*K*) for the applied sample and a desirable settling time (15 s). Some 57% of the stationary phase was retained in the coil. First, the coiled column was entirely filled with the stationary phase (upper phase). Then, the apparatus was rotated forward at 800 rpm, while the mobile phase (lower phase) was pumped into the column in a tail to head (T → H) direction at a flow rate of 1.7 mL/min. After the mobile phase front emerged and the hydrodynamic equilibrium was established in the column, about 10 mL of filtered sample solution (357 mg, dissolved in 5 mL of upper phase and 5 mL of lower phase) was injected through the injection module. The effluent of the column was continuously monitored by TLC on silica gel, with a mobile phase of EtOAc/AcOH/H_2O (10:2:3, v/v/v). The spots were observed under ultraviolet light (254 nm). A total of 150−160 fractions of 8 mL each were collected and pooled together according to the TLC pattern (**Figure 1**).

Structural Identification of the Compounds. Nuclear magnetic resonance (NMR) spectra were obtained using a Bruker spectrometer, operated at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Dimethyl sulfoxide (DMSO-d_6) and methanol (CD_3OD) were used as solvents. The UV spectra were obtained using a Heλios α V-3.06 UV/vis spectrophotometer; MeOH was used as solvent. HPLC−DAD was used for the identification and quantification of the main compounds in the crude extracts, fractions, and as purity criteria for the isolated compounds before NMR measurements and assays.

Isolated Compounds. The following compounds were identified by comparison of its ^{1}H and ^{13}C NMR data with literature and are in agreement with the proposed structures. Isoorientin, homoorientin (**1**) (*6*−*8*); isoscoparin (**2**) (*9*, *10*); swertiajaponin, 7-*O*-methyl-6-*β*-D-glucopyranosylluteolin, 7-*O*-methylisoorientin (**3**) (*11*, *12*); isoorientin, 2″-*O*-rhamnoside (**4**) (*7*); orientin (**5**) (*6*); chlorogenic acid (**6**) (*13*); and caffeic acid (**7**) were identified by UV spectrum and TLC comparison with a standard sample. The structures of compounds **1**−**7** are presented in **Figure 2**. The ^{1}H NMR spectroscopic data of compounds **1**−**6** and ^{13}C NMR data of compounds **1**−**4** can be ordered directly from the authors.

HPLC Analysis. The determination of flavonoids in the samples was performed according to Sánchez-Rabaneda et al. (*14*) with some modifications. HPLC analysis was performed using HPLC−DAD Merck-Hitachi (LaChrom, Tokio, Japan) equipment consisting of a L-7100 pump, a L-7455 UV diode array detector, and a D-7000 chromatointegrator. A 250 × 4.6 mm i.d., 5 μm C18-RP column (Phenomenex, Torrence, CA) was used. The compounds were monitored at 256 nm, and the absorbance was measured between 200 and 400 nm. Gradient elution was carried out with water/0.1% formic acid (solvent A) and 20% solvent A in 80% acetonitrile (solvent B) at a constant flow rate of 1 mL/min. A linear gradient was used (*14*). Under our experimental conditions, R_t (min) of the isolated compounds was as follows: compound **1** (44.2 min); compound **2** (48.8 min); compound **3** (44.5 min); compound **4** (43.8 min); compound **5** (44.9 min); compound **6** (36.3 min); and compound **7** (41.3 min).

Calibration curves were performed to estimate the main active compound content in the samples. The correlation between concentration/peak area was assessed by the ordinary least-squares regression model. The amount of the active principles was expressed as g per 100 g of dry material.

DPPH Decoloration Assay. The free radical scavenging effect of the extracts and compounds was assessed by the decoloration of a methanolic solution of DPPH as previously reported (*15*). Crude extracts were assayed at 100, 33, and 11 μg/mL, while pure compounds were assayed at 10, 3.3, and 1.1 μg/mL. The scavenging of free radicals by extracts and compounds was evaluated spectrophotometrically at 517 nm against the absorbance of the DPPH radical.

The percentage of decoloration was calculated as follows:

$$\%\ \text{of decoloration} = 1 - \frac{\text{absorbance of compound/extract}}{\text{absorbance of blank}} \times 100$$

The degree of decoloration indicates the free radical scavenging efficiency of the substances. For extracts, values are reported as mean ± SD of three determinations. For the compounds, the half-maximal inhibitory concentration (IC_{50}) was calculated by linear regression analysis and expressed as mean of three determinations. Quercetin was used as a free radical scavenger reference compound.

Superoxide Anion. The enzyme xanthine oxidase (XO) is able to generate the superoxide anion by oxidation of reduced products from

intracellular ATP metabolism. In this reaction, the XO oxidizes the substrate hypoxanthine generating superoxide anion, which reduces the nitrobluetetrazolium dye (NBT), leading to a chromophore with absorption maxima at 560 nm. Superoxide anion scavengers reduce the generation speed of the chromophore. The activity was measured spectrophotometrically as reported previously (*15*) using a Genesys-10 UV scanning spectrophotometer. Extracts were evaluated at 50 μg/mL and compounds were evaluated at 50, 25, and 10 μg/mL. Values are presented as mean ± SD of three determinations. The activity of the pure compounds is presented as mean IC_{50}, calculated by linear regression analysis. The IC_{50} value corresponds to the concentration of the compound that reduces in 50% the scavenging activity. Quercetin was used as a reference compound. The percentage of superoxide anion scavenging effect was calculated as follows:

$$\%\ \text{of scavenging activity} = \frac{E - S}{E} \times 100$$

where E = A − B and S = C − (B + D). A is the optical density of the control; B is the optical density of the control blank; C is the optical density of the sample; and D is the optical density of the sample blank.

Inhibition of Xanthine Oxidase (XO). The XO activities with xanthine as the substrate were measured spectrophotometrically using a Genesys-10 UV scanning spectrophotometer as previously reported (*15*). Allopurinol was used as a standard inhibitor of XO. Extracts were evaluated at 50 μg/mL, and the results are presented as mean ± SD of three determinations.

The percentage of XO inhibition was calculated as follows:

$$\%\ \text{of inhibition} = \frac{(\text{control} - \text{control blank}) - (\text{sample} - \text{sample blank})}{(\text{control} - \text{control blank})} \times 100$$

Lipoperoxidation in Erythrocytes. Studies on erythrocyte lipid peroxidation were carried out as previously described (*15*). Human red blood cells obtained from healthy donors were washed three times in cold phosphate buffered saline (PBS) by centrifugation at 11 000*g* for 5 min. After the last washing, cells were resuspended in PBS, and their density was adjusted to 1 mM haemoglobin in each reaction tube. The final cell suspensions were incubated with different concentrations of the test compounds dissolved in DMSO and PBS during 5 min at 37 °C. The final concentration of DMSO in the samples and controls was 1%. After incubation, cells were exposed to *t*-butylhydroperoxide (1 mM) for 15 min at 37 °C under vigorous shaking. After treatment, lipid peroxidation was determined indirectly by the TBARs formation as described previously (*15*). Results are expressed as percentages of inhibition as compared to controls. The percentage of inhibition of the formation of TBARs (% inhibition of lipid peroxidation) was calculated as follows:

$$\%\ \text{inhibition of lipid peroxidation} = \frac{(A_1 - A_t)}{(A_1 - A_2)} \times 100$$

where A_1, A_2, and A_t are the absorbance values at 535 nm for the unprotected samples, the blanks, and the test samples, respectively.

Cytotoxicity Assay. The cytotoxic effect of the compounds, expressed as cell viability, was assessed on a permanent fibroblast cell line derived from human lung (MRC-5) (ATCC no. CCL-171). MRC-5 fibroblasts were grown as monolayers in minimum essential Eagle medium (MEM), with Earle's salts, 2 mM L-glutamine, and 1.5 g/L sodium bicarbonate, supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 μg/mL streptomycin in a humidified incubator with 5% CO_2 in air at 37 °C. Cells were plated at a density of 2.5×10^3 per well in 96-well plates. Confluent cultures of MRC-5 cells were treated with medium containing the compounds at concentrations ranging from 80 to 1000 μM. The substance was first dissolved in DMSO and then in MEM. The final concentration of DMSO in the test medium and controls was 1%. Cells were exposed for 24 h to test medium with or without the compound (control). Each drug concentration was tested in quadruplicate and repeated three times in separate experiments. At the end of the incubation, the neutral red uptake (NRU) assay was carried out as described by Rodríguez and Haun (*16*). To calculate the IC_{50} values, the results were transformed to a percentage of controls, and the IC_{50} values were graphically obtained from the dose−response curves. The IC_{50} value is defined as the concentration of the compound that reduces in 50% cell viability.

Statistical Analysis. To determine whether there was any difference between activity or phenolic content of samples, variance analysis using an Anova test was applied. Values of $P < 0.05$ were considered as significantly different. The differences among means were determined using the Tukey's multiple comparison test. To assess the relationship between the activities and the phenolic content, Pearson's correlation coefficients were calculated with 95% confidence. The Statistical Package S-Plus 2000 for Windows was used to analyze the data.

RESULTS

The different *C. citratus* extracts presented free radical scavenging effects in the DPPH and superoxide anion assays, with values ranging between 40 and 68% and 15−32% at 33 and 50 μg/mL, respectively. In the DPPH assay, the IC_{50} values for the CH_2Cl_2, EtOAc, and polar fractions were 152, 131, and 41 μg/mL, respectively (**Figure 1**). The extracts were also active in inhibiting lipid peroxidation in erythrocytes at 500 μg/mL, with values in the range of 19−71%, but none was active inhibiting the xanthine oxidase at 50 μg/mL. The most active extract in all the assays was the MeOH extract (**Table 1**).

A correlation analysis was used to assess the relationship between the flavonoid content with the antioxidant activity (DPPH) of the polar extracts. A significant correlation was found with a correlation coefficient of $r = 0.873$; $P < 0.05$ (**Figure 3**). Furthermore, the correlation between total flavonoid content and the DPPH scavenging activity presented a determination coefficient of $R^2 = 0.762$. This result suggested that 76% of the free radical scavenging activity of the lemongrass extracts resulted from the contribution of flavonoids.

From the methanolic extract of *C. citratus*, the following compounds were isolated and identified by spectroscopic methods. *C*-Glycosylflavonoids: isoorientin **1**, isoscoparin **2**, swertiajaponin **3**, isoorientin 2″-*O*-rhamnoside **4**, orientin **5**, chlorogenic acid **6**, and caffeic acid **7**. Isoscoparin **2**, swertiajaponin **3**, and orientin **5** are reported for the first time for *C. citratus*. The content of the isolated phenolics in the different extracts is summarized in **Table 2**. None of the compounds proved to be cytotoxic toward human lung fibroblasts even at concentrations as high as 1 mM.

As shown by TLC and HPLC analysis, the main free radical scavengers and antioxidants occurring in *C. citratus* are present in the water infusion and decoction as well as in the MeOH and MeOH/H_2O extracts. There are, however, significant differences both in the relative proportions as well as in the total content of compounds **1**−**7** in the extracts.

DISCUSSION

In a survey of leaf flavonoids from *Gramineae*, Harborne and Williams (*17*) reported the occurrence of tricin and flavone *C*-glycosides in five *Cymbopogon* species, while luteoforol was found in two of them, and sulfated flavonoids as well as apigiforol were found in only one species. The plant material used for the survey included Australian and Asian *Cymbopogon* collections. While both flavones, flavonoids *C*- and *O*-glycoside, were reported by De Matouschek and Stahl-Biskup (*7*) from a Peruvian sample of *C. citratus*, the plant growing in Chile contained only *C*-glycosylflavonoids in detectable amounts.

Table 1. Percentage of Activity Relative to the Corresponding Control Induced by *C. citratus* Extracts and Compounds on Free Radical and Superoxide Anion Scavenging and Inhibition of Lipid Peroxidation

samples	extraction yield % w/w	decoloration of DPPH (%)	scavenging of the superoxide anion (%)	inhibition of lipoperoxidation (%)
Extracts		33 μg/mL	50 μg/mL	500 μg/mL
MeOH/H_2O 7:3	21.5 ± 1.4	57.8 ± 4.8	22.5 ± 0.6	19.1 ± 3.1
MeOH/H_2O 1:1	22.6 ± 2.2	55.8 ± 0.6	21.8 ± 0.2	38.1 ± 5.0
MeOH	18.2 ± 1.2	67.9 ± 2.5	32.4 ± 0.1	71.5 ± 2.9
decoction	17.6 ± 0.9	41.9 ± 4.7	15.2 ± 0.5	41.6 ± 5.1
infusion	21.2 ± 1.3	40.2 ± 0.4	27.1 ± 0.4	47.3 ± 4.7
Compounds		IC_{50} (μM)	IC_{50} (μM)	100 (μg/mL)
1 isoorientin		9.1	52.9	71.3 ± 8.3
2 isoscoparin		39.6	52.3	41.7 ± 4.0
3 swertiajaponin		13.4	110.3	60.6 ± 8.3
4 isoorientin 2-*O*-rhamnoside		17.5	84.4	60.6 ± 9.9
5 orientin		10.0	79.0	68.7 ± 6.9
6 chlorogenic acid		13.8	54.2	33.8 ± 4.2
7 caffeic acid		9.4	68.8	84.9 ± 0.4
quercetin[a]		9.1	72.0	80.7 ± 1.0

[a] Reference compound. Results are presented as mean ± SD.

Table 2. Flavonoids and Chlorogenic and Caffeic Acid Content of *C. citratus* Extracts as Percent w/w Yields in Terms of Dry Starting Material[a]

	% (w/w)				
	MeOH/H_2O (7:3)	MeOH/H_2O (1:1)	MeOH	decoction	infusion
Compound					
1 isoorientin	0.16 ± 0.02	0.15 ± 0.03	0.47 ± 0.04	traces	0.08 ± 0.04
2 isoscoparin	traces	0.07 ± 0.02	0.09 ± 0.03	traces	traces
3 swertiajaponin	0.77 ± 0.04	0.72 ± 0.01	0.58 ± 0.01	0.37 ± 0.05	0.29 ± 0.02
4 isoorientin-2-*O*-rhamnoside	0.68 ± 0.03	1.16 ± 0.02	0.67 ± 0.02	0.37 ± 0.02	0.37 ± 0.03
5 orientin	0.44 ± 0.02	0.35 ± 0.01	0.44 ± 0.05	0.14 ± 0.03	0.08 ± 0.02
6 chlorogenic acid	traces	0.09 ± 0.01	traces	traces	0.35 ± 0.04
7 caffeic acid	traces	0.13 ± 0.03	0.03 ± 0.01	0.07 ± 0.01	0.10 ± 0.02

[a] Infusion: 10 g of air-dried, powdered plant material soaked in 250 mL of boiling water for 15 min. Decoction: 10 g of air-dried, powdered material boiled in 250 mL of water for 2.5 h.

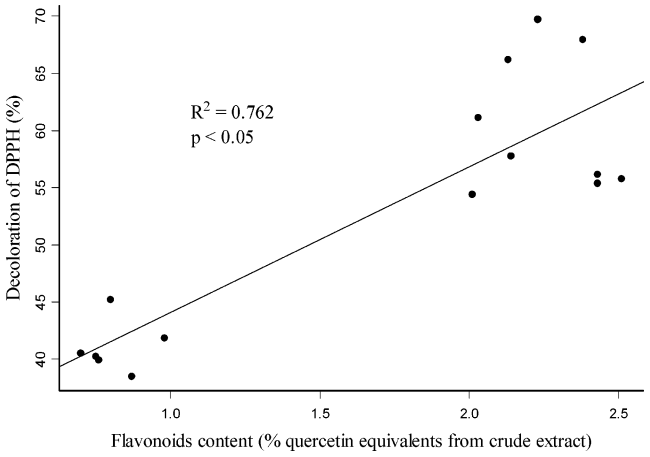


Figure 3. Correlation between antioxidant/DPPH activity (decoloration % at 33 μg/mL) vs total flavonoid content.

The antioxidant activity of *C*-glycosylflavones including isoorientin and orientin has been reported by several authors. Budzianowski et al. (*18*) compared the antioxidant effect of 10 *C*-glycosylflavonoids and found that only isoorientin and orientin were active. According to Huguet et al. (*19*), isoorientin and orientin are potent superoxide anion scavengers. In a study on the antioxidants of *Crotalaria sessiliflora*, Mum'im et al. (*20*) assessed the effect of isoorientin and orientin with the scavenging of the free radical DPPH and linoleic acid oxidation. Both compounds displayed a strong antiperoxidative activity with an IC_{50} value of 9.5 μM in the DPPH assay. Orientin behaved as an inhibitor of the lipid peroxidation induced by $FeSO_4$ and cysteine in rat liver microsomes (*21*) and was able to protect human lymphocyte chromosomes from the damage produced by γ-radiation. The radioprotection appears to be associated with the antioxidant activity of the compound. Orientin also showed a significant inhibition of TBAR formation in the 12.5−20 μM range (*22*).

Isoorientin-6″-*O*-glucoside proved to be a water-soluble antioxidant from *Gentiana arisanensis* assessed by the DPPH test and scavenges the superoxide anion in a concentration-dependent manner (*23*). Isoorientin-2″-*O*-rhamnoside from *Allophyllus edulis* var. *gracilis* was described as a hepatoprotective compound (*24*). The hepatoprotective effect of isoorientin isolated from *Gentiana olivieri* has been reported previously (*25*). Chlorogenic and caffeic acid are well-known plant antioxidants.

Isoorientin (**1**) and orientin (**5**) presented similar activities toward the DPPH with IC_{50} values about 9−10 μM. Methoxylation at C-4′ reduces the activity as shown by compound **2**. The sugar position at C-6 or C-8 did not influence the effect as can be seen by comparing the activity of compounds **1** and **5**. The activity for the diglycoside **4** was lower than that for the monoglycoside **1**. The best superoxide anion scavengers were isoorientin **1** and isoscoparin **2** with the sugar attached at C-6. An additional sugar at C-6, methoxylation at C-7, or glycosidation at C-8 instead of C-6 reduced the superoxide anion scavenger effect of the reported compounds. In the inhibition of lipid peroxidation at 100 μg/mL, compounds **1** and **5** presented similar effects, reducing peroxidation by 69−71%. Methoxylation at C-4′ lowers the effect, while methoxylation

at C-7 or the presence of a diglycoside at C-6 led to compounds inhibiting peroxidation by 60%. The best antioxidant effect found in the methanol extract can be related with the isoorientin (0.47%) and orientin content (0.44%) of the sample, the former compound being present in much higher concentrations than in the methanol/water and aqueous extracts.

Lemongrass infusions have been shown to be devoid of toxic effects in human volunteers as well as being inactive as a hypnotic or anxiolytic (*26*). The study was performed with doses similar to those employed in traditional medicine. A weak diuretic and antiinflammatory effect was reported for the oral intake of a 10 or 20% decoction at a dose of 25 mL/kg in rats (*27*). Previous studies undertaken with the ethanol extract of lemongrass indicated antimutagenic activity in various models (*28*). It is thus conceivable that lemongrass contains some components that may be cancer chemopreventive. In fact, the extract has been shown to inhibit rat colon carcinogenesis in animal models (*29*).

The main compounds obtained from the plant in our study are the *C*-glycosylflavones orientin and isoorientin as well as chlorogenic acid. Recently, isoorientin and chlorogenic acid have been shown to display hypoglycaemic effects in streptozotocin diabetic rats (*30*). The content and free radical scavenging/antioxidant activity of the extracts and main compounds of *C. citratus* as well as the absence of cytotoxicity at doses up to 1 mM support the reputed beneficial properties of the tea and soft drinks prepared from lemongrass.

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