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# *Pterospartum tridentatum*, *Gomphrena globosa* and *Cymbopogon citratus*: A phytochemical study focused on antioxidant compounds

Custódio Lobo Roriz [a], Lillian Barros [a,*], Ana Maria Carvalho [a], Celestino Santos-Buelga [b], Isabel C.F.R. Ferreira [a,**]

[a] Mountain Research Centre (CIMO), ESA, Polytechnic Institute of Bragança, Campus de Santa Apolónia, Apartado 1172, 5301-855 Bragança, Portugal
[b] GIP-USAL, Faculty of Pharmacy, University of Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain

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ABSTRACT

*Pterospartum tridentatum* (L.) Willk., *Gomphrena globosa* L. and *Cymbopogon citratus* (DC) Stapf. are examples of medicinal plants that demand a more detailed characterization. Therefore, phenolic composition (e.g., phenolic acids and flavonoids) was analyzed by chromatographic and mass spectrometry techniques and the antioxidant activity was also accessed through free radicals scavenging activity, reducing power and inhibition of lipid peroxidation in brain homogenates.

*C. citratus* revealed the highest β-carotene bleaching and lipid peroxidation inhibitions, being luteolin 2″-*O*-rhamnosyl-6-*C*-glucoside the main compound. *P. tridentatum* presented the highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and reducing power and mainly dihydroflavonol and isoflavone derivatives were detected. Otherwise, *G. globosa* presented kaempferol 3-*O*-rutinoside as the most abundant phenolic compound and betacyanins were only present in this sample. It is very interesting to study the phytochemical composition of these plants, given the importance of their consumption.



## 1. Introduction

Free radicals are produced in natural metabolism of aerobics cells, mostly in the form of oxygen reactive species (ROS) (Ferreira, Barros, & Abreu, 2009). Oxidative stress is a serious imbalance between the generation of ROS and antioxidant protection in favor of the former, causing excessive oxidative damage (Halliwell, 2011). In fact, the non-controlled production of free radicals can be related not only to various chronic diseases such as cancer, cardiovascular and neurodegenerative diseases, but also to the aging process (Ferreira et al., 2009).

Antioxidant species that can be generated internally can counteract the high amounts of ROS. Nevertheless, despite its high efficiency, the endogenous defenses are not enough, being necessary to obtain antioxidants through diet, in order to maintain the values of free radicals at low levels, so that the antioxidant defense systems of the body is not compromised (Carocho & Ferreira, 2013). One of the ways to get antioxidants through the diet is by incorporating a high variety of vegetables and fruits. Plants are full of antioxidants (e.g., phenolic compounds), because they are subject to severe oxidative stress as they produce oxygen during photosynthesis (Halliwell, 2012).

*Pterospartum tridentatum* (L.) Willk., *Gomphrena globosa* L. and *Cymbopogon citratus* (DC.) Stapf. could be explored as sources of antioxidant phytochemicals. *P. tridentatum*, plant similar to a broom, is a species from the Fabaceae family that grows spontaneously in thermo-Mediterranean conditions of the Iberian Peninsula and North Africa (Carvalho, 2010). There are countless purposes for which this species is used, among them are the treatment of diseases of the respiratory system and of type 2 diabetes (Vitor et al., 2004). *G. globosa*, known as globe amaranth, is a plant native from Brazil, Panama and Guatemala from the Amaranthaceae family usually recommended to treat respiratory system diseases (Cai, Xing, Sun, & Corke, 2006), diabetes, jaundice, hypertension, urinary system conditions, as well as kidney and prostate problems (Dinda et al., 2006; Lans, 2007). *C. citratus* is a tropical plant of the Poaceae family from Southeast Asia, commonly referred as lemongrass. Studies previously conducted reported hypoglycemic, hipolipidemic, anxiolytic and sedative effects (Adeneye & Agbaje, 2007; Blanco, Costa, Freire, Santos, & Costa, 2009), and various other uses for inflammation, diabetes, and nervous disorders.

Some researchers have studied wild samples of *P. tridentatum* regarding antioxidant properties (Coelho, Gonçalves, Alves, & Martins, 2011; Pinela, Barros, Carvalho, & Ferreira, 2011; Vitor et al., 2004) and composition in phenolic compounds (Paulo et al., 2008; Vitor et al., 2004), commercial samples of *G. globosa* concerning betacyanins and phenolic compounds (Ferreres, Gil-Izquierdo, Valentão, & Andrade, 2011; Silva et al., 2012), and wild and commercial samples of *C. citratus* regarding antioxidant properties and phenolic composition (Cheel, Theoduloz, Rodríguez, & Schmeda-Hirschmann, 2005; Figueirinha, Paranhos, Pérez-Alonso, Santos-Buelga, & Batista, 2008; Koh, Mokhtar,

* Corresponding author. Tel.: +351 273 303219; fax: +351 273 325405.
** Corresponding author. Tel.: +351 273 303200; fax: +351 273 325405.
*E-mail addresses:* lillian@ipb.pt (L. Barros), iferreira@ipb.pt (I.C.F.R. Ferreira).

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& Iqbal, 2012). Nevertheless, as far as we know, this is the first study reporting antioxidant properties and a detailed characterization in phenolic compounds of certified commercial samples from Portugal, obtained according sustainable harvesting and organic farming principles.

## 2. Materials and methods

### 2.1. Samples

Plant material of *P. tridentatum* (L.) Willk., *G. globosa* L. and *C. citratus* (DC) Stapf. was purchased from Ervital, a Portuguese company from Castro Daire (Portugal). This company, settled in a high diverse mountain region (Montemuro, a Natura 2000 site), markets several certified plant materials with different origin, such as sustainable wild harvesting of spontaneous local species and organic farming of exogenous species. *P. tridentatum* flowers were wild gathered in spring 2012 (respecting plant phenology and abundance) and the other studied species were grown, also in 2012, with organic farming methods. Harvested plants were processed using in-storage and low temperature drying methods (solar heated air, average daily temperature around 30–32 °C in shade conditions and controlled relative humidity). Samples for analysis were prepared from dried plant materials provided by the company, and botanical identification was confirmed by Ana Maria Carvalho, responsible of the medicinal plant collection of the Herbarium of the Escola Superior Agrária (BRESA), of the Polytechnic Institute of Bragança (Trás-os-Montes, Portugal).

### 2.2. Standards and reagents

HPLC-grade acetonitrile was obtained from Merck KgaA (Darmstadt, Germany) and was purchased from Fisher Scientific (Lisbon, Portugal). Formic and acetic acids were purchased from Prolabo (VWR International, France). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Matreya (Pleasant Gap, PA, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Phenolic standards were from Extrasynthèse (Genay, France). Water was treated in Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

### 2.3. Phenolic compounds composition

Phenolic compounds were determined by High-Performance Liquid Chromatography (HPLC, Hewlett-Packard 1100, Agilent Technologies, Santa Clara, CA, USA) as previously described by the authors (Barros et al., 2013). Double online detection was carried out in the diode array detector (DAD) using 280 nm and 370 nm as preferred wavelengths and in a mass spectrometer (MS) connected to the HPLC system via the DAD cell outlet. The phenolic compounds were identified by comparing their retention time, UV–vis and mass spectra with those obtained from standard compounds, when available. Otherwise, peaks were tentatively identified comparing the obtained information with available data reported in the literature. For quantitative analysis, a calibration curve for each available phenolic standard was constructed based on the UV signal. For the identified phenolic compounds for which a commercial standard was not available, the quantification was performed through the calibration curve of other compounds from the same phenolic group. The results were expressed in μg per g of dry weight.

#### 2.3.1. Betacyanins

Each sample (1 g) was extracted with 30 mL of methanol containing 0.5% trifluoroacetic acid (TFA), and filtered through a Whatman No. 4 paper. The residue was then re-extracted twice with additional 30 mL portions of 0.5% TFA in methanol. The combined extracts were evaporated at 35 °C to remove the methanol, and re-dissolved in water. For purification, the extract solution was deposited onto a C-18 SepPak® Vac 3 cc cartridge (Phenomenex), previously activated with methanol followed by water; sugars and more polar substances were removed by passing through 10 mL of water and betalain/betacyanin pigments were further eluted with 5 mL of methanol:water (80:20, *v*/*v*) containing 0.1% TFA. The extract was concentrated under a vacuum, was lyophilized, was re-dissolved in 1 mL of 20% aqueous methanol and was filtered through a 0.22-μm disposable LC filter disk for HPLC analysis. Betacyanins were determined by HPLC as previously described by the authors (using anthocyanins analysis methodology; Guimarães et al., 2013). Double detection was carried out by DAD, using 520 nm as the preferred wavelength, and in a MS connected to the HPLC system via the DAD cell outlet. The betacyanins were tentatively identified by comparing their UV–vis and mass spectra with available data information reported in the literature and expressed as relative percentage (%) of their areas recorded at 520 nm.

### 2.4. Evaluation of antioxidant activity

#### 2.4.1. Extracts preparation

The methanolic extracts were obtained from the plant material. Each sample (1 g) was extracted by stirring with 25 mL of methanol (25 ºC at 150 rpm) for 1 h and subsequently filtered through Whatman No. 4 paper. The residue was then extracted with 25 mL of methanol (25 °C at 150 rpm) for 1 h. The combined methanolic extracts were evaporated at 40 °C (rotary evaporator Büchi R-210, Flawil, Switzerland) to dryness.

#### 2.4.2. Antioxidant activity assays

DPPH radical-scavenging activity was evaluated by using an ELX800 microplate reader (Bio-Tek Instruments, Inc.; Winooski, VT, USA), and calculated as a percentage of DPPH discolouration using the formula: $[(A_{DPPH} - A_S) / A_{DPPH}] \times 100$, where $A_S$ is the absorbance of the solution containing the sample at 515 nm, and $A_{DPPH}$ is the absorbance of the DPPH solution. Reducing power was evaluated by the capacity to convert $Fe^{3+}$ into $Fe^{2+}$, measuring the absorbance at 690 nm in the microplate reader mentioned above. Inhibition of β-carotene bleaching was evaluated though the β-carotene/linoleate assay; the neutralization of linoleate free radicals avoids β-carotene bleaching, which is measured by the formula: β-carotene absorbance (after 2 h of assay/initial absorbance) × 100. Lipid peroxidation inhibition in porcine (*Sus scrofa*) brain homogenates was evaluated by the decrease in thiobarbituric acid reactive substances (TBARS); the color intensity of the malondialdehyde-thiobarbituric acid (MDA-TBA) was measured by its absorbance at 532 nm; the inhibition ratio (%) was calculated using the following formula: $[(A - B) / A] \times 100\%$, where A and B were the absorbance of the control and the sample solution, respectively The results were expressed in $EC_{50}$ values (sample concentration providing 50% of antioxidant activity or 0.5 of absorbance in the reducing power assay) and trolox was used as the standard (Barros et al., 2013).

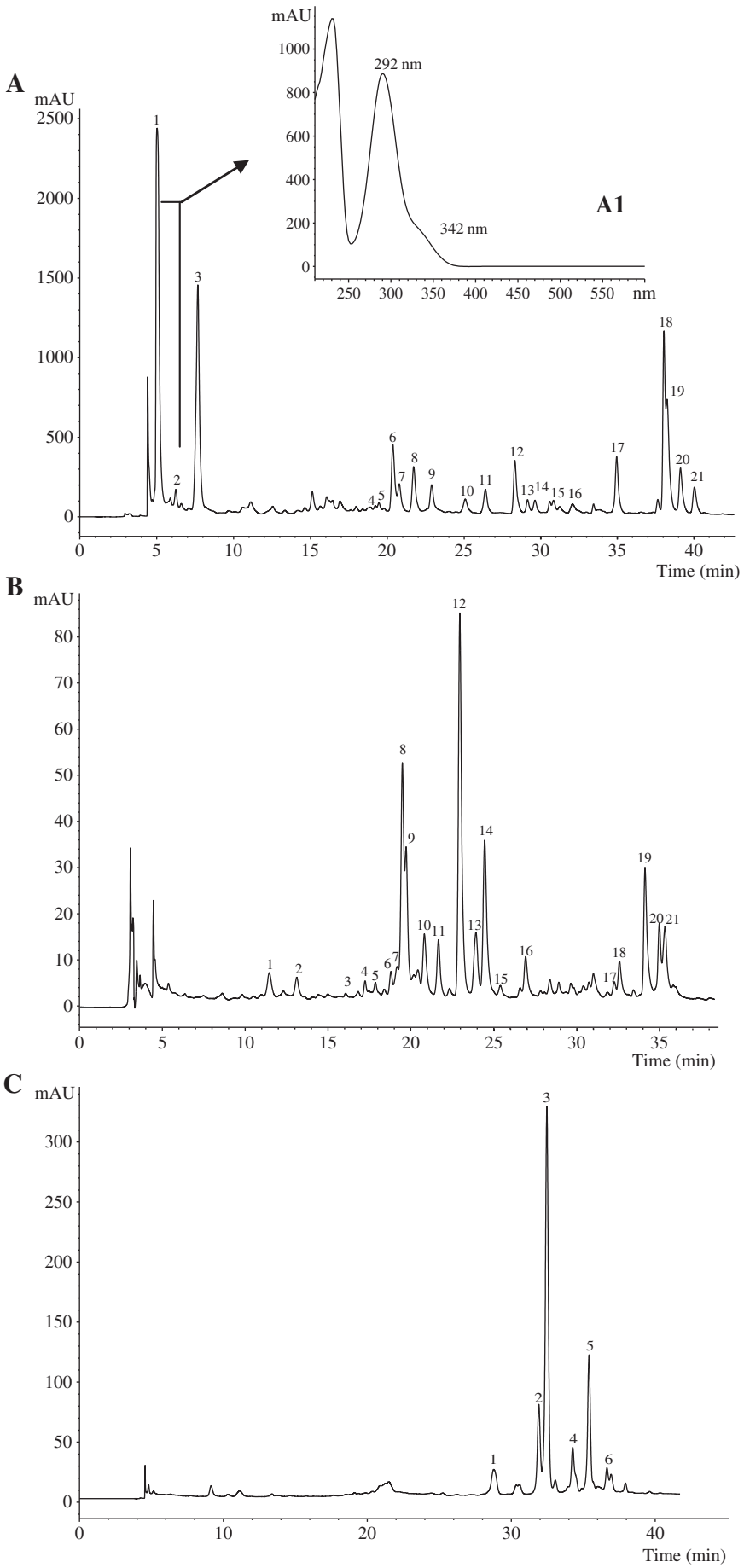
### 2.5. Statistical analysis

For each species, three samples were analyzed and all the assays were carried out in triplicate, and the results are expressed as mean values and standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$. This treatment was carried out using the SPSS v.22.0 program.

## 3. Results and discussion

### 3.1. Phenolic compounds characterization

The phenolic compound profiles of *G. globosa* (Gg), *C. citratus* (Cc) and *P. tridentatum* (Pt) are shown in Figs. 1 and 2. Data (retention time, λmax in the visible region, molecular ion and main fragment ions observed in $MS^2$) obtained by HPLC–DAD–ESI/MS analysis regarding


A
mAU
Time (min)
A1
292 nm
342 nm
nm
B
mAU
Time (min)
C
mAU
Time (min)

phenolic compounds and betacyanins, identification of compounds and individual quantification are presented in Tables 1–3. 5-*O*-Caffeoylquinic acid (peak $1^{Cc}$), caffeic acid (peak $2^{Cc}$), *trans*-*p*-coumaric acid (peak $9^{Cc}$; peak $4^{Gg}$), *trans*-ferulic acid (peak $7^{Gg}$), isorhamnetin 3-*O*-rutinoside (peak $13^{Gg}$), isorhamnetin 3-*O*-glucoside (peak $15^{Gg}$), genistein (peak $12^{Pt}$), kaempferol 3-*O*-rutinoside (peak $12^{Gg}$), kaempferol 3-*O*-glucoside (peak $14^{Gg}$), luteolin 6-*C*- glucoside (isoorientin, peak $7^{Cc}$), luteolin 7-*O*-glucoside (peak $13^{Cc}$), luteolin (peak $18^{Cc}$), quercetin 3-*O*-rutinoside (peak $8^{Gg}$ and peak $5^{Pt}$) and quercetin 3-*O*-glucoside (peak $10^{Gg}$ and peak $6^{Pt}$) were positively identified according to their retention, mass and UV–vis characteristics by comparison with commercial standards.

Twenty-one flavonoids were detected in *P. tridentatum* (Table 1). Peaks $1^{Pt}$ and $2^{Pt}$ presented the same pseudomolecular ion $[M-H]^-$ at *m/z* 465, and their UV spectra (Fig. 1A) and main $MS^2$ fragments point to they could be dihydroflavonol *C*-glycosyl derivatives. Thus, ions at *m/z* 375 and 345 would result, respectively, from the losses of 90 mu and 120 mu, characteristics of *C*-attached hexoses and due to partial cleavage of the glycosyl residue (Cuyckens & Claeys, 2004). Fragments at *m/z* 447, 357 and 327 could be explained by the loss of $H_2O$ (−18 mu) from the original compound and the mentioned ions, respectively, probably by cleavage of the −OH at position C-3 of the flavonoid. The ion at *m/z* 317 may be due to the loss of CO (−28 mu) from the majority fragment at *m/z* 345, and the fragment at *m/z* 167 could correspond to the $^{0,2}A_0^-$ ion from the cleavage of the aglycone. The observation of Ferreres, Silva, Andrade, Seabra, and Ferreira (2003) that the loss −90 mu (ion *m/z* at 375) is unusual in 8-*C*-hexoses allowed tentatively assigning peaks 1 and 2 as 6-*C*-hexosides. All in all, the compounds were tentatively identified as dihydroquercetin 6-*C*-hexosides, and they might be speculated to be two stereoisomers due to the asymmetric nature of C2 and C3 of dihydroquercetin. As far as we know, dihydroflavonol *C*-glycosides, namely different dihydroquercetin 6-*C*-glucoside isomers, have only been reported in two natural sources: *Ulmus wallichiana* (family Ulmaceae; Rawat, Manmeet, Kunal, Naibedya, & Rakesh, 2009) and *Paepalanthus argenteus* (Eriocaulaceae; Dokkedal, Lavard, Santos, & Vilegas, 2007). Therefore, this would be the first report to this type of unusual compounds in Fabaceae.

Peak $3^{Pt}$ presented a pseudomolecular ion $[M-H]^-$ at *m/z* 479, yielding a product ion at *m/z* 359 by loss of −120 mu, characteristic of *C*-hexosyl flavones, whereas fragments at *m/z* 341, 221, and 167 are compatible with an ortho-trihydroxylated B ring of a flavonol and a 5,7-dihydroxy A ring (Fabre, Rustan, Hoffmann, & Quetin-Leclercq, 2001; Wu, Yan, Li, Liu, & Liu, 2004). The compound was tentatively identified as myricetin 6-*C*-glucoside, already described in *P. tridentatum* by Paulo et al. (2008). Peaks $4^{Pt}$ and $7^{Pt}$ presented UV spectra with λmax 352–356 nm and an $MS^2$ product ion at *m/z* 301, indicating that they correspond to quercetin derivatives. According to their pseudomolecular ions, they were assigned as quercetin *O*-deoxyhexosyl-hexoside ($[M-H]^-$ at *m/z* 609) and quercetin *O*-hexoside ($[M-H]^-$ at *m/z* 463).

The remaining phenolic compounds were identified as isoflavone derivatives based on their characteristic UV spectra and mass fragmentation patterns. Peaks $8^{Pt}$, $10^{Pt}$ and $12^{Pt}$ were identified as genistein derivatives. Peak $8^{Pt}$, with a pseudomolecular ion $[M-H]^-$ at *m/z* 431 releasing a fragment at *m/z* 269 ($[M-162]^-$, (loss of a glycosyl moiety) was tentatively associated with genistein 7-*O*-glucoside (genistin), owing to its previous description in *P. tridentatum* (Paulo et al., 2008; Vitor et al., 2004), although the nature and position of the glycosyl moiety could not be established in our case. Peak $10^{Pt}$ ($[M-H]^-$ at *m/z* 431) released two $MS^2$ fragment ions at *m/z* 311 and 269, corresponding to the losses of 120 and 42 mu, characteristic of *C*-hexosyl flavones. This compound was tentatively assigned as genistein 8-*C*-glucoside, previously reported in *Genista tenera* by Rauter et al. (2005) and in other Fabaceae species (Talhi & Silva, 2012). Peak $12^{Pt}$ ($[M-H]^-$ at *m/z* 269) would correspond to genistein aglycone.

A compound with the same pseudomolecular ion and fragmentation characteristics as peak $9^{Pt}$ was isolated from *P. tridentatum* and fully identified by Vitor et al. (2004) as 5,5′-dihydroxy-3′-methoxyisoflavone-7-*O*-β-glucoside, so that the compound herein detected was associated with this structure.

Peaks $14^{Pt}$, $15^{Pt}$, $16^{Pt}$ and $17^{Pt}$ were identified as biochanin A derivatives according to their UV and mass spectra characteristics. Peak $15^{Pt}$, with a pseudomolecular ion $[M-H]^-$ at *m/z* 431 releasing a fragment at *m/z* 283 ($[M-H-162]^-$, loss of a glycosyl moiety) was tentatively assigned as sissotrin (i.e., biochanin A 7-*O*-glucoside) owing to its previous identification in *P. tridentatum* flowers (Paulo et al., 2008; Vitor et al., 2004). Peak $14^{Pt}$ with a pseudomolecular ion $[M-H]^-$ at *m/z* 607, and fragment ions resulting from the consecutive losses of two hexosyl residues (*m/z* at 445 and 283) was identified as a biochanin A *O*-hexoside-*O*-hexoside. Similarly, peak $16^{Pt}$ ($[M-H]^-$ at *m/z* 649), 42 mu greater than $14^{Pt}$ could be assigned as biochanin A *O*-acetylhexoside-*O*-hexoside. As far as we know, these compounds have not been reported in *P. tridentatum*.

Peaks $18^{Pt}$ and $19^{Pt}$ presented the same pseudomolecular ion $[M-H]^-$ at *m/z* 283, coherent with a methylgenistein. They were tentatively identified as prunetin (7-*O*-methylgenistein), previously reported in *P. tridentatum* (Paulo et al., 2008), and biochanin A (4′-*O*-methylgenistein), owing to the presence of other biochanin A derivatives in the analyzed sample. Peak $21^{Pt}$ ($[M-H]^-$ at *m/z* 297), 14 mu greater than peaks $18^{Pt}$ and $19^{Pt}$ could be associated with a methyl derivative of prunetin or biochanin A. Peak $20^{Pt}$ presented a pseudomolecular ion $[M-H]^-$ at *m/z* 299 releasing a fragment at *m/z* 284 (−15 mu, loss a methyl group), compatible with a trihydroxymethoxy-isoflavonoid. A compound with similar characteristics was reported in *P. tridentatum* by Paulo et al. (2008) and assigned to a 7-*O*-methylorobol.

No definite structures could be matched for peaks $11^{Pt}$, $13^{Pt}$ and $17^{Pt}$. This latter presented a pseudomolecular ion $[M-H]^-$ at *m/z* 491, 46 mu greater than peak $15^{Pt}$, which might be explained as due to a formic acid adduct, whose formation has been discussed in literature (de Rijke, Zappey, Ariese, Gooijer, & Brinkman, 2003, 2004). Thus, it could be speculated to correspond to an artifact (formic acid adduct of sissotrin) formed under the experimental conditions used. Similar speculation could be made for peak $11^{Pt}$ (ion $[M-H]^-$ at *m/z* 505) that might correspond to a formic acid adduct of methylprunetin or methylbiochanin A *O*-hexoside. As for peak $13^{Pt}$ with a pseudomolecular ion $[M-H]^-$ at *m/z* 341 and fragments at *m/z* 298 (−43 mu) and 283 (−43–15 mu), it could be only speculated to be a methylprunetin or methylbiochanin A derivative.

Dihydroflavonol *C*-derivatives (namely peak $1^{Pt}$, 3873.55 μg/g dw) were the major compounds found in *P. tridentatum* (Table 1). Paulo et al. (2008) and Vitor et al. (2004) studied a wild sample of *P. tridentatum* from Portugal and presented some similarities in the phenolic composition. However, those authors only detected up to nine compounds of the groups of flavanols and isoflavones, but they did not report dihydroflavonol derivatives and did not present quantification results. The identification of dihydroflavonol *C*-derivatives in our samples is particularly important, not only as they are majority phenolic compounds, but also for their possible biological activity. Indeed, the dihydroflavonol *C*-hexosides identified in *Ulmus wallichiana* were found to possess relevant in vitro osteogenic activity being able to promote osteoblast differentiation in primary cultures of rat osteoblasts, making them good candidates to be used in osteoporosis therapy (Rawat et al., 2009).

Twenty-seven phytochemicals were detected in *G. globosa*, six of which were phenolic acid derivatives, fifteen flavonoids, mainly flavonol derivatives (Fig. 1B), and six betacyanins (Table 2, Fig. 1C). Peaks

**Fig. 1.** HPLC phenolic profiles of (A)—*P. tridentatum* (recorded at 280 nm); (A1)—UV spectra of peaks 1 and 2; (B)—*G. globosa* (recorded at 370 nm); (C)—*G. globosa* betacyanin profile (recorded at 520 nm).

$3^{Gg}$ and $5^{Gg}$ were assigned as the *cis* isomers of *p*-coumaric acid and ferulic acid, whereas the corresponding *trans* isomers (i.e., peaks $4^{Gg}$ and $7^{Gg}$) were confirmed by comparison with standards, as described above. The *trans* isomers of these phenolic acids were also found in the inflorescences of *G. globosa* (Silva et al., 2012). Peaks $1^{Gg}$ and $2^{Gg}$ with the same pseudomolecular ion $[M-H]^-$ at *m/z* 355 and releasing

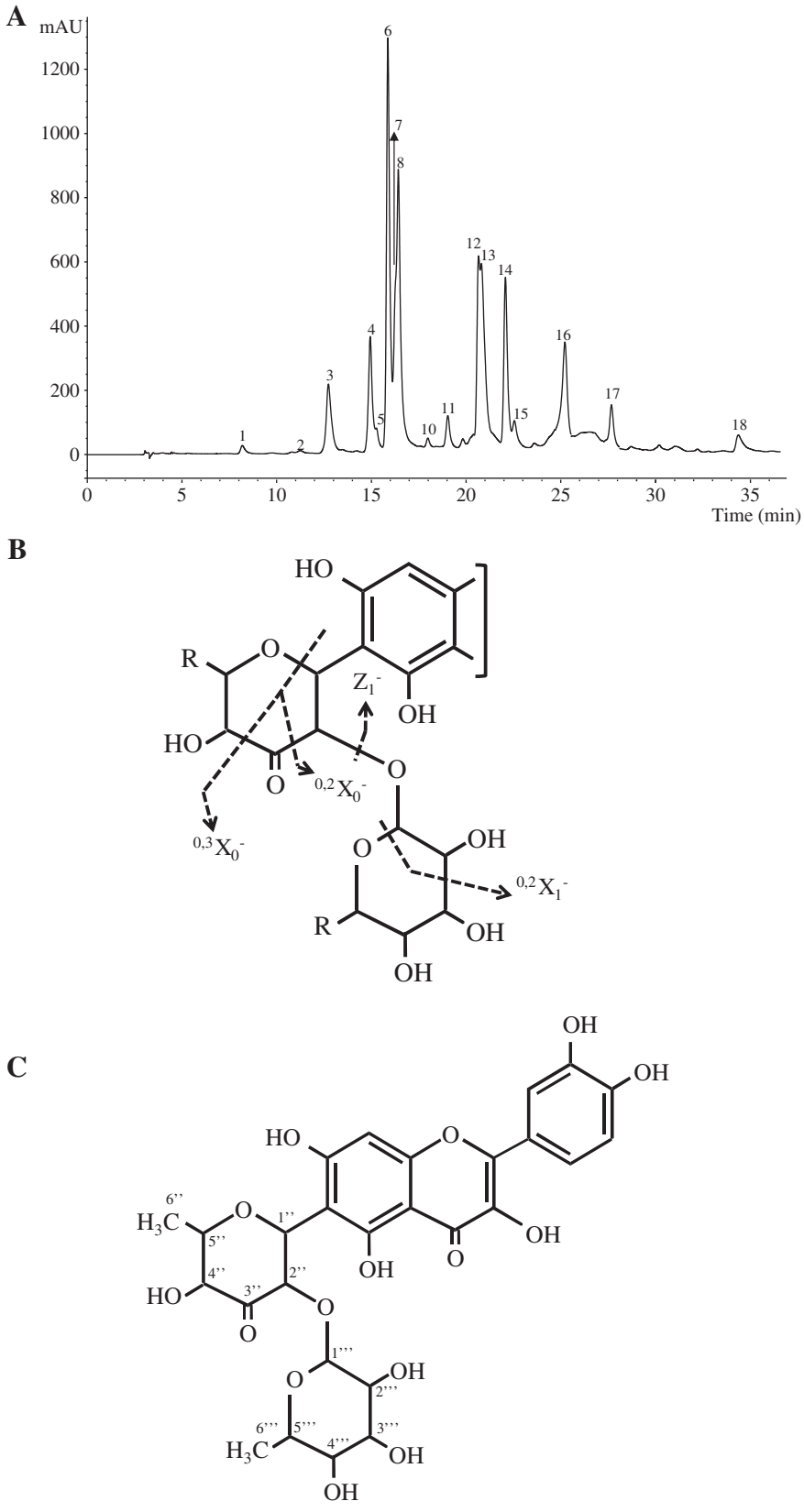



**Fig. 2.** (A) HPLC phenolic profiles of *C. citratus* (recorded at 370 nm); (B) General fragmentation of *O*-glycosyl-*C*-glycosyl flavonoids (based on Ferreres, Gil-Izquierdo, Andrade, Valentao, & Tomás-Barberán, 2007); (C) Chemical structure of 2″-*O*-deoxyosyl-6-*C*-(6-deoxy-*pento*-hexos-ulosyl) present in *C. citratus*.

**Table 1**
Retention time (Rt), wavelengths of maximum absorption in the visible region ($\lambda_{max}$), mass spectral data, identification and quantification of phenolic compounds in *P. tridentatum* (mean ± SD).

| Peak | Rt (min) | $\lambda_{max}$ (nm) | Molecular ion $[M-H]^-$ (*m/z*) | Main $MS^2$ fragments (*m/z*) | Tentative identification | Quantification (μg/g dw) |
|---|---|---|---|---|---|---|
| $1^{Pt}$ | 5.03 | 292, sh342 | 465 | 447(25), 375(79), 357 (21), 345(100), 327(86), 317(42), 167(63) | Dihydroquercetin 6-*C*-hesoxide | 3873.55 ± 34.12 |
| $2^{Pt}$ | 6.24 | 294, sh348 | 465 | 447(7), 375(54), 357(13), 345(100), 327(50), 317(23), 167(38) | Dihydroquercetin 6-*C*-hesoxide | 130.77 ± 3.09 |
| $3^{Pt}$ | 7.67 | 290, sh340 | 479 | 359(100), 341(7), 221(5), 167(8) | Myricetin-6-*C*-glucoside | 1316.66 ± 2.33 |
| $4^{Pt}$ | 19.22 | 352 | 609 | 463(4), 301(46) | Quercetin deoxyhexosyl-hexoside | 100.13 ± 1.50 |
| $5^{Pt}$ | 19.45 | 358 | 609 | 301(100) | Quercetin-3-*O*-rutinoside | 125.67 ± 1.30 |
| $6^{Pt}$ | 20.36 | 356 | 463 | 301(100) | Quercetin-3-*O*-glucoside (isoquercitrin) | 963.19 ± 4.29 |
| $7^{Pt}$ | 20.77 | 356 | 463 | 301(100) | Quercetin *O*-hexoside | 474.68 ± 2.82 |
| $8^{Pt}$ | 21.71 | 260, sh332 | 431 | 269(100) | Genistein 7-*O*-glucoside (genistin) | 162.86 ± 2.97 |
| $9^{Pt}$ | 22.88 | 260, sh336 | 461 | 446(35), 341(5), 299(88), 283(29) | 5,5′-Dihydroxy-3′-methoxy-isoflavone-7-*O*-β-glucoside | 94.14 ± 0.93 |
| $10^{Pt}$ | 25.07 | 260, sh332 | 431 | 311(5), 269(30) | Genistein-8-*C*-glucoside | 57.73 ± 1.04 |
| $11^{Pt}$ | 26.38 | 256, sh322 | 505 | 459(3), 297(100), 282(22) | Methylbiochanin A/methylprunetin *O*-hexoside | 89.71 ± 0.52 |
| $12^{Pt}$ | 28.29 | 260, sh334 | 269 | 241(4), 225(6), 201(5), 181(2), 133(7) | Genistein | 167.11 ± 3.43 |
| $13^{Pt}$ | 29.12 | 262, sh336 | 341 | 298(89), 283(35) | Methylbiochanin A/methylprunetin derivative | 40.29 ± 1.53 |
| $14^{Pt}$ | 29.61 | 258, sh330 | 607 | 445(4), 283(100) | Biochanin A *O*-hexoside-*O*-hexoside | 44.84 ± 1.04 |
| $15^{Pt}$ | 32.04 | 260, sh340 | 445 | 283(100) | Biochanin A 7-*O*-glucoside (sissotrin) | 53.22 ± 0.19 |
| $16^{Pt}$ | 33.41 | 260, sh336 | 649 | 607(11), 445(3), 283(100) | Biochanin A *O*-acetylhexoside-*O*-hexoside | 25.67 ± 0.26 |
| $17^{Pt}$ | 34.91 | 260, sh332 | 491 | 445(3), 283(100) | Biochanin A *O*-hexoside | 201.48 ± 2.82 |
| $18^{Pt}$ | 37.99 | 262, sh332 | 283 | 268(100), 239(7), 224(5), 195(2), 135(2) | 4′-*O*-Methylgenistein (biochanin A) | 433.53 ± 1.97 |
| $19^{Pt}$ | 38.19 | 262, sh334 | 283 | 268(100) | 7-*O*-Methylgenistein (prunetin) | 372.62 ± 2.61 |
| $20^{Pt}$ | 39.07 | 262, sh338 | 299 | 284(100), 281(4), 256(4), 241(2), 228(5), 148(2) | 7-*O*-methylorobol | 167.80 ± 1.30 |
| $21^{Pt}$ | 39.97 | 260, sh294 | 297 | 282(100) | Methylbiochanin A/methylprunetin | 105.37 ± 1.78 |
| | | | | | Total flavonols | 2980.33 ± 9.24 |
| | | | | | Total dihydroflavonols | 4004.32 ± 31.03 |
| | | | | | Total isoflavone | 2016.37 ± 5.52 |
| | | | | | Total flavonoids | 9001.01 ± 16.28 |

Tr–traces; dw–dry weight.

an $MS^2$ fragment at *m/z* 193 ([ferulic acid-H]$^-$) from the loss of a hexosyl moiety (−162 mu) were tentatively assigned as *cis* and *trans* ferulic acid hexoside, respectively.

Peaks $9^{Gg}$, $11^{Gg}$, $16^{Gg}$, $20^{Gg}$ and $21^{Gg}$ were identified as kaempferol derivatives, based on their UV spectra and the production of an $MS^2$ product ion at *m/z* 285. Similarly, peaks $6^{Gg}$ and $17^{Gg}$ ($MS^2$ product ion at *m/z* 301) were assigned as quercetin derivatives. Peaks $9^{Gg}$ and $11^{Gg}$ presented pseudomolecular ions [M-H]$^-$ at *m/z* 725 and 579 and $MS^2$ fragments at *m/z* 593 and 447 ([M-H-132]$^-$, loss of a pentosyl moiety), respectively, which further lost a rutinosyl (−308 mu; peak $9^{Gg}$) or a hexosyl moiety ([M-H-162]$^-$; peak $11^{Gg}$) to yield the aglycone fragment at *m/z* 285. Compounds with the same pseudomolecular ions were also found in extracts of *G. globosa* inflorescences by Ferreres et al. (2011) and Silva et al. (2012), and identified as kaempferol 3-*O*-(2-pentosyl, 6-*O*-rhamnosyl)-hexoside and kaempferol 3-*O*-(2-pentosyl)-hexoside, respectively, so that these structures were assumed for the compounds detected in our sample. Similarly, peak $6^{Gg}$, with a pseudomolecular ion [M-H]$^-$ at *m/z* 595 and an $MS^2$ fragment at *m/z* 301 ([M-H-132-162]$^-$ loss of pentosyl and hexosyl moieties). A compound with the same pseudomolecular ion was also found by Ferreres et al. (2011) in *G. globosa* inflorescences, which was identified as quercetin 3-*O*-(2-pentosyl)-hexoside. In those cases, the assignment of the substitution position of the pentose was based on the observation by the authors of fragment ions from the loss of the pentosyl residue (−132 mu) and of pentosyl + water (−150 mu), characteristic of such an interglycosidic linkage (Cuyckens, Rozenberg, Hoffmann, & Claeys, 2001). In our case, no fragment ion resulting from the loss of the pentosyl residue was noticed, which would suggest that it was linked at position 6″ of the hexose, so that peak $6^{Gg}$ was tentatively assigned as quercetin 3-*O*-(6-pentosyl)-hexoside.

A compound with the same characteristics as peak $20^{Gg}$ ([M-H]$^-$ at *m/z* 593, $MS^2$ fragment ion at *m/z* 285 from the loss of rhamnosyl and hexosyl moieties) was also identified by Ferreres et al. (2011) and Silva et al. (2012) in *G. globosa* inflorescences and assigned as kaempferol 3-*O*-(6-rhamnosyl)-hexoside. The observation in our case of an $MS^2$ fragment ion at *m/z* 447 from the loss of the rhamnosyl residue might indicate its location at position 2″ of the hexose, so that the compound was tentatively identified as kaempferol 3-*O*-(2-rhamnosyl)-hexoside. Peak $16^{Gg}$ was associated with a kaempferol *O*-acetylhexoside according to its pseudomolecular ion [M-H]$^-$ at *m/z* 489 and $MS^2$ fragment released at *m/z* 285 ([M-H-42-162]$^-$, loss of acetyl and hexosyl moieties).

Peaks $17^{Gg}$ ([M-H]$^-$ at *m/z* 639) and $21^{Gg}$ ([M-H]$^-$ at *m/z* 623) should correspond to quercetin and kaempferol derivatives bearing glucuronyl and hexosyl moities. In both cases the observation of $MS^2$ fragments resulting from the alternative losses of each residue (i.e., −176 and −162 mu) might suggest that each sugar was located on a different position of the aglycone. Therefore, these compounds were tentatively assigned as quercetin *O*-glucuronide-*O*-hexoside (peak $17^{Gg}$) and kaempferol *O*-glucuronide-*O*-hexoside (peak $21^{Gg}$).

Peaks $18^{Gg}$ and $19^{Gg}$ ([M–H]$^-$ at *m/z* 475 and 517 mu, respectively) originated a base peak at *m/z* 313 mu, which could correspond to a trihydroxy-methylenedioxyflavone, probably gomphrenol (3,5,4′-trihydroxy-6,7-methylenedioxyflavone) early described in *G. globosa* leaves (Bouillant, Redolfi, Cantisani, & Chopin, 1978). Peaks with the same pseudomolecular ions were detected in *G. globosa* inflorescences by Ferreres et al. (2011) and Silva et al. (2012) and suggested to correspond to gomphrenol-3-*O*-hexoside and gomphrenol-3-*O*-(6-acetyl)-hexoside, so that those identities were also tentatively assumed in our case.

Compounds $22^{Gg}$ to $27^{Gg}$ were identified as betacyanin derivatives (Table 2) already described in *G. globosa* (Fig. 1C); no anthocyanins were found together with the betacyanins, which is in agreement with the previous reports (Cai, Sun, & Corke, 2001; Cai et al., 2006; Ferreres et al., 2011; Kugler, Stintzing, & Carle, 2007; Silva et al., 2012). These pigments would belong to the (iso)gomphrenin-type betacyanins

**Table 2**
Retention time (Rt), wavelengths of maximum absorption in the visible region ($\lambda_{max}$), mass spectral data, identification, quantification of phenolic compounds and relative percentage of betacyanins in *G. globosa* (mean ± SD).

| Peak | Rt (min) | $\lambda_{max}$ (nm) | Molecular ion $[M-H]^-$ (*m/z*) | Main $MS^2$ fragments (*m/z*) | Tentative identification | Quantification (μg/g dw) |
|---|---|---|---|---|---|---|
| $1^{Gg}$ | 11.43 | 326 | 355 | 193(100) | *cis*-Ferulic acid hexoside | 20.41 ± 0.76 |
| $2^{Gg}$ | 13.10 | 326 | 355 | 193(100) | *trans*-Ferulic acid hexoside | 15.13 ± 0.57 |
| $3^{Gg}$ | 16.07 | 312 | 163 | 119(100) | *cis*-*p*-Coumaric acid | 1.09 ± 0.16 |
| $4^{Gg}$ | 16.98 | 312 | 163 | 119(100) | *trans*-*p*-Coumaric acid | 5.92 ± 0.66 |
| $5^{Gg}$ | 18.44 | 322 | 193 | 178(16), 134(100), 117(3) | *cis*-Ferulic acid | 7.95 ± 0.27 |
| $6^{Gg}$ | 18.79 | 360 | 595 | 301(100) | Quercetin 3-*O*-(6-pentosyl)-hexoside | 2.93 ± 0.25 |
| $7^{Gg}$ | 19.17 | 324 | 193 | 178(35), 134(100), 117(2) | *trans*-Ferulic acid | 27.73 ± 0.44 |
| $8^{Gg}$ | 19.48 | 354 | 609 | 301(100) | Quercetin 3-*O*-rutinoside | 23.76 ± 0.17 |
| $9^{Gg}$ | 19.71 | 344 | 725 | 593(5), 285(30) | Kaempferol 3-*O*-(2-pentosyl, 6-*O*-rhamnosyl)-hexoside | 16.71 ± 0.21 |
| $10^{Gg}$ | 20.81 | 358 | 463 | 301(100) | Quercetin 3-*O*-glucoside | 8.67 ± 0.70 |
| $11^{Gg}$ | 21.67 | 350 | 579 | 447(5), 285(50) | Kaempferol 3-*O*-(2-pentosyl)-hexoside | 7.67 ± 0.18 |
| $12^{Gg}$ | 22.95 | 350 | 593 | 285(100) | Kaempferol 3-*O*-rutinoside | 48.44 ± 0.16 |
| $13^{Gg}$ | 23.93 | 354 | 623 | 315(100) | Isorhamnetin 3-*O*-rutinoside | tr |
| $14^{Gg}$ | 24.46 | 348 | 447 | 285(100) | Kaempferol 3-*O*-glucoside | 18.81 ± 0.43 |
| $15^{Gg}$ | 25.40 | 354 | 477 | 315(100) | Isorhamnetin 3-*O*-glucoside | tr |
| $16^{Gg}$ | 26.93 | 350 | 489 | 285(100) | Kaempferol *O*-acetylhexoside | 4.89 ± 0.04 |
| $17^{Gg}$ | 32.27 | 340 | 639 | 463(32), 301(34) | Quercetin *O*-glucuronide-*O*-hexoside | 1.64 ± 0.15 |
| $18^{Gg}$ | 32.59 | 274,340 | 475 | 313(100) | Gomphrenol 3-*O*-hexoside | 3.87 ± 0.22 |
| $19^{Gg}$ | 34.14 | 274,340 | 517 | 313(100) | Gomphrenol 3-*O*-(6-acetyl)-hexoside | 14.18 ± 0.37 |
| $20^{Gg}$ | 34.99 | 352 | 593 | 447(9), 285(60) | Kaempferol 3-*O*-(2-rhamnosyl)-hexoside | 8.68 ± 0.23 |
| $21^{Gg}$ | 35.34 | 348 | 623 | 447(4), 285(23) | Kaempferol *O*-glucuronide-*O*-hexoside | 11.25 ± 0.08 |
| | | | | | Total phenolic acids | 78.23 ± 2.32 |
| | | | | | Total flavonoids | 171.50 ± 2.62 |
| | | | | | Total phenolic compounds | 249.73 ± 4.95 |
| Peak | Rt (min) | $\lambda_{max}$ (nm) | Molecular ion $[M+H]^+$ (*m/z*) | Main $MS^2$ fragments (*m/z*) | Identification | Relative percentage (%) |
| $22^{Gg}$ | 28.79 | 550 | 697 | 551(2), 389(22) | Gomphrenin II | 7.66 ± 0.56 |
| $23^{Gg}$ | 31.91 | 550 | 697 | 551(3), 389(39) | Gomphrenin II | 12.99 ± 0.41 |
| $24^{Gg}$ | 32.48 | 550 | 727 | 551(4), 389(41) | Gomphrenin III | 50.21 ± 0.94 |
| $25^{Gg}$ | 34.27 | 550 | 697 | 551(2), 389(21) | Isogomphrenin II | 6.13 ± 0.11 |
| $26^{Gg}$ | 35.40 | 546 | 727 | 551(4), 389(38) | Isogomphrenin III | 17.23 ± 0.24 |
| $27^{Gg}$ | 36.65 | 500 | 683 | 507(2), 345(22) | 17-Descarboxy-amaranthin | 4.10 ± 0.11 |

tr—traces; dw—dry weight.

(substituted at *C*-6 of betanidin/isobetanidin), differing from betanin-type betacyanins (substituted at *C*-5 of betanidin/isobetanidin) (Cai et al., 2001, 2006; Heuer, Wray, Metzger, & Strack, 1992). The mass differences of 146 mu (*m/z* 697–551) and 176 mu (*m/z* 727–551) indicated the presence of aromatic acyl groups (i.e., coumaroyl and feruloyl) at the *C*-6 of glucose in gomphrenins/isogomphrenins. Thus, peaks $22^{Gg}$, $23^{Gg}$ and $25^{Gg}$ were identified as gomphrenin II/isogomphrenin II, based on the observation of two main fragments at *m/z* 551, loss of a *p*-coumaroyl group (−146 mu) and at *m/z* 389, further loss of a hexosyl moiety (−162 mu). For peaks $24^{Gg}$ and $26^{Gg}$ a pseudomolecular ion $[M+H]^+$ at *m/z* 727, was observed, so that they were identified as gomphrenin III/isogomphrenin III. The main fragments at *m/z* 551 and 389 indicated the loss of a feruloyl group (−176 mu) and the further loss of a hexosyl moiety (−162 mu), respectively. The later elution of peaks $25^{Gg}$ and $26^{Gg}$ allowed their identification as isogomphrenins II and III, respectively. Finally, peak $27^{Gg}$ was identified as decarboxylated amaranthin ($[M+H]^+$ at *m/z* 683), previously reported in red petals and in flowers of *G. globosa* (Kugler et al., 2007). Those authors indicated that the higher retention time and the hypsochromic shift of the maximum UV spectra (around 33 nm) as compared to amaranthin (betanidin 5-*O*-β-glucuronosylglucoside), suggested a 17-descarboxy structure. Similar observation was described in the literature for 17-descarboxybetanin from red beet (Stintzing, Trichterborn, & Carle, 2006) and erect spiderling (Stintzing et al., 2004).

Flavonoids were the main phenolic compounds found in *G. globosa* being kaempferol 3-*O*-rutinoside (peak $12^{Gg}$, 48.44 μg/g dw) the main flavonol (Table 2). Gomphrenin III (peak $24^{Gg}$, 50.21%) was the major betacyanidin found (Table 2). Silva et al. (2012) and Ferreres et al. (2011) presented a slightly different profile in the samples studied by them, presenting flavonol (quercetin, kaempferol and isorhamnetin derivatives) and gomphrenol derivatives as the main phenolic compounds. They also reported the presence of eight betacyanins, although with a different profile of that found in our samples. Furthermore, Silva et al. (2012) showed higher values in their quantification results for all the compounds identified. Kugler et al. (2007) and Cai et al. (2001, 2006) presented a more complex identification of betacyanins in petals of *G. globosa*.

Eighteen phenolic compounds were identified in *C. citratus* (Table 3, Fig. 2A). But for three hydroxycinnamoyl derivatives (peaks $1^{Cc}$, $2^{Cc}$ and $9^{Cc}$) the rest of peaks corresponded to flavone derivatives, which were identified based on the fragmentation patterns described for *C*- and *O*-glycosyl flavones by Ferreres et al., 2003; Ferreres, Llorach, & Gil-Izquierdo, 2004; Ferreres et al., 2007 (Fig. 2B). From them, only two peaks ($12^{Cc}$ and $13^{Cc}$) were found to be *O*-glycosylated on the aglycone. Peak $13^{Cc}$ was positively identified as luteolin 7-*O*-glucoside by comparison with a standard, whereas peak $12^{Cc}$, with a pseudomolecular ion $[M-H]^-$ at *m/z* 593 releasing two fragment ions at *m/z* 447 ($[M-H-146]^-$, loss of a deoxyhexosyl moiety) and at *m/z* 285 ($[M-H-162]^-$, further loss of an hexosyl moiety), was assigned as luteolin 7-*O*-neohesperoside, based on the previous identification of this compound in *C. citratus* leaves by Figueirinha et al. (2008).

Peak $3^{Cc}$ ($[M-H]^-$ at *m/z* 579) and peaks $4^{Cc}$ and $5^{Cc}$ (both with $[M-H]^-$ at *m/z* 563) presented a fragmentation pattern characteristic of asymmetric di-*C*-glycosides (Ferreres et al., 2003). The fragments at *m/z* 489 and 473 ($[(M-H)-90]^-$) and 459 and 443 ($[(M-H)-120]^-$) indicated the presence of a *C*-hexosyl unit. For peak $3^{Cc}$, fragments showing the loss of 60 mu, typical of pentosyl units, were observed at *m/z* 519 ($[(M-H)-60]^-$), 399 ($[(M-H)-120-60]^-$) and 369 ($[(M-H)-120-90]^-$); and similarly occurred for peaks $4^{Cc}$ and $5^{Cc}$ (fragment at *m/z* 503; $[(M-H)-60]^-$). For peak $3^{Cc}$, the observation of a base peak at *m/z* 459 ($[(M-H)-120]^-$; partial loss of a hexosyl moiety) and its high abundance in relation to that at *m/z* 519 ($[(M-H)-60]^-$; partial loss of a pentosyl

**Table 3**
Retention time (Rt), wavelengths of maximum absorption in the visible region ($\lambda_{max}$), mass spectral data, identification and quantification of phenolic compounds in *C. citratus* (mean ± SD).

| Peak | Rt (min) | $\lambda_{max}$ (nm) | Molecular ion $[M-H]^-$ (*m/z*) | Main $MS^2$ fragments (*m/z*) | Tentative identification | Quantification (μg/g dw) |
|---|---|---|---|---|---|---|
| $1^{Cc}$ | 8.19 | 324 | 353 | 191(100), 179(4), 161(5), 135(3) | 5-*O*-Caffeoylquinic acid | 101.56 ± 1.56 |
| $2^{Cc}$ | 11.28 | 326 | 179 | 135(100) | Caffeic acid | 21.27 ± 0.79 |
| $3^{Cc}$ | 12.73 | 350 | 579 | 561(10), 519(6), 489(56), 459(100), 399(52), 369(47) | Luteolin 6-*C*-hexosyl-8-*C*-pentoside | 97.33 ± 3.75 |
| $4^{Cc}$ | 14.94 | 336 | 563 | 545(27), 503(40), 473(100), 443(83), 383(90), 353(85) | Apigenin 6-*C*-pentosyl-8-*C*-hexoside | 246.70 ± 4.82 |
| $5^{Cc}$ | 15.26 | 336 | 563 | 545(20), 503(37), 473(100), 443(83), 383(73), 353(83) | Apigenin 6-*C*-pentosyl-8-*C*-hexoside | 31.75 ± 0.50 |
| $6^{Cc}$ | 15.87 | 350 | 593 | 473(100), 429(37), 357(26), 339(12), 309(20), (35) | Luteolin 2″-*O*-deoxyhexosyl-6-*C*-glucoside | 2138.07 ± 32.61 |
| $7^{Cc}$ | 16.24 | 350 | 447 | 429(30), 357(100), 339(15), 327(86), 297(22), 285(15) | Luteolin 6-*C*-glucoside | 93.93 ± 0.47 |
| $8^{Cc}$ | 16.43 | 350 | 549 | 531(18), 489(36), 459(100), 441(25), 429(21), 399(55), 369(52) | Luteolin 6-*C*-pentosyl-8-*C*-pentoside | 270.05 ± 7.47 |
| $9^{Cc}$ | 16.96 | 310 | 163 | 119(100) | *trans*-*p*-Coumaric acid | 32.83 ± 0.88 |
| $10^{Cc}$ | 17.98 | 350 | 549 | 531(12), 489(32), 459(100), 441(18), 429(18), 399(76), 369(60) | Luteolin 6-*C*-pentosyl-8-*C*-pentoside | 20.00 ± 1.02 |
| $11^{Cc}$ | 19.04 | 344 | 577 | 457(26), 413(100), 341(15), 311(14), 293(77) | Apigenin 2″-*O*-deoxyhexosyl-*C*-hexoside | 127.70 ± 6.59 |
| $12^{Cc}$ | 20.66 | 350 | 593 | 447(6), 285(22) | Luteolin 7-*O*-neohesperoside | 926.42 ± 15.71 |
| $13^{Cc}$ | 20.81 | 348 | 447 | 285(100) | Luteolin 7-*O*-glucoside | 1410.24 ± 37.12 |
| $14^{Cc}$ | 22.07 | 352 | 563 | 503(3), 473(100), 417(17), 399(53), 357(23), 327(25), 298(40) | Luteolin 2″-*O*-deoxyhexosyl-*C*-pentoside | 1029.80 ± 41.40 |
| $15^{Cc}$ | 22.55 | 350 | 417 | 399(42), 357(100), 339(19), 327(90), 311(7), 297(35) | Luteolin 6-*C*-pentoside | 38.53 ± 1.29 |
| $16^{Cc}$ | 25.22 | 350 | 575 | 531(33), 429(38), 411(100), 367(65), 357(15), 337(20), 309(10) | Luteolin 2″-*O*-deoxyosyl-6-*C*-(6-deoxy-*pento*-hexos-ulosyl) | 1122.62 ± 7.15 |
| $17^{Cc}$ | 27.67 | 350 | 577 | 487(10), 473(40), 413(100), 371(15), 323(27) | Methyl-luteolin 2″-*O*- deoxyhexosyl-6-*C*-hexoside. | 58.63 ± 0.95 |
| $18^{Cc}$ | 34.37 | 350 | 285 | 175(14), 151(18), 133(32) | Luteolin | 187.18 ± 1.35 |
| | | | | | Total phenolic acids | 155.65 ± 1.47 |
| | | | | | Total flavonoids | 7798.96 ± 70.79 |
| | | | | | Total phenolic compounds | 7954.61 ± 69.32 |

dw—dry weight.

moiety), suggested that the hexose was located at position 6 of the aglycone. Conversely, for peaks $4^{Cc}$ and $5^{Cc}$, the base peak at *m/z* 473 ([(M-H)-90]$^-$) and the high abundance of the fragment at *m/z* 503 ([(M-H)-60]$^-$) would indicate a 6-*C*-pentosyl unit. The ions at *m/z* 369 and 353 [aglycone + 83]$^-$ and 399 and 383 [aglycone + 113]$^-$, supported the conclusion that luteolin and apigenin, respectively, were the aglycones, which allowed the identification as a luteolin 6-*C*-hexoside-8-*C*-pentoside (peak $3^{Cc}$) and apigenin 6-*C*-pentoside-8-*C*-hexoside (peak $4^{Cc}$ and $5^{Cc}$). Peak $15^{Cc}$ presented a pseudomolecular ion $[M-H]^-$ at *m/z* 417, 30 mu lower than peak $7^{Cc}$ (positively identified as luteolin-6-*C*-glucoside by comparison with a standard) suggesting a pentosyl unit bound to the aglycone, which together with the fragments at *m/z* 357 ([(M-H)-60]-; base peak) and 327 ([(M-H)-90]-) allowed its tentative identification as a luteolin 6-*C*-pentoside. Peaks $8^{Cc}$ and $10^{Cc}$ presented the same UV spectra and pseudomolecular ion $[M-H]^-$ at *m/z* 549. Their $MS^2$ fragmentation suggested the presence of two pentosyl units linked at positions 6 and 8, so that they could be identified as luteolin 6-*C*-pentoside-8-*C*-pentoside. The existence of two peaks should be explained by different substituting pentoses in each case. The presence of all the previous peaks in *C. citratus* leaves was also reported by Figueirinha et al. (2008).

Peaks $11^{Cc}$ showed a pseudomolecular ion $[M-H]^-$ at *m/z* 577, releasing five $MS^2$ fragments ions. The loss of 120 mu (ion at *m/z* 457) is characteristic of a *C*-hexosyl flavone, whereas the fragment at *m/z* 413 ([M-H-146-18]$^-$) would indicate a deoxyhexose *O*-glycosylated on the hydroxyl group at position 2″ of the *C*-glycosylating sugar (Ferreres et al., 2007). The other three product ions at *m/z* 341 ([aglycone + 71)]$^-$), *m/z* 311 ([aglycone + 41)]$^-$) and *m/z* 293 ([aglycone + 41-18]$^-$) are usual in mono-*C*-glycosyl derivatives *O*-glycosylated on 2″ position (Ferreres et al., 2007, 2011). Thus, this peak could be tentatively identified as apigenin 2″-*O*-deoxyhexosyl-*C*-hexoside. Similar reasoning can be applied for the assignment of peak $6^{Cc}$ ($[M-H]^-$ at *m/z* 593). The base peak at *m/z* 473 (loss of 120 mu) indicated a *C*-hexosyl flavone, and the fragment at *m/z* 429 ([M-H-146-18]$^-$) would be characteristic of the *O*-glycosylation at position 2″ of the *C*-attached sugar (Ferreres et al., 2007). Ions at *m/z* 357 ([aglycone + 71)]$^-$), *m/z* 339 ([aglycone + 71-$H_2O$)]$^-$) and *m/z* 309 ([aglycone + 41-$H_2O$]$^-$) confirmed luteolin as aglycone. Therefore, the peak was tentatively identified as luteolin 2″-*O*-deoxyhexosyl-6-*C*-hexoside, also reported in *C. citratus* by Figueirinha et al. (2008). Peak $14^{Cc}$ showed a pseudomolecular ion $[M-H]^-$ at *m/z* 563, 30 mu lower than peak $6^{Cc}$. The observation of a weak ion at *m/z* 417 ([(M-H)-146]$^-$) and a major fragment at *m/z* 399 ([(M-H)-146-18]$^-$) indicated an *O*-linked deoxyhexose, and the observation of ions at *m/z* 503 ([(M-H)-60]-) and 473 ([(M-H)-90]-) revealed a pentose directly linked to the aglycone. This suggested the identification of this peak as a luteolin 2″-*O*-deoxyhexosyl-*C*-pentoside.

Peak $17^{Cc}$ showed a pseudomolecular ion $[M-H]^-$ at *m/z* 577. In the $MS^2$ fragmentation, the observation of a $[M-H-90]^-$ ion (*m/z* at 487) and the lack of a $[M-H-60]^-$ ion suggested a *C*-attached hexose, whereas the main fragment at *m/z* 413 ([(M-H)-146-18]$^-$) indicated an *O*-linked deoxyhexose; the loss of −104 mu to give rise to the fragment at *m/z* 473 could be interpreted as corresponding to the partial fragmentation of the deoxyhexose ($^{0,\ 2}X_1^-$ ion), whereas fragments at *m/z* 371 ([aglycone + 71]$^-$) and 323 ([aglycone + 41-$H_2O$]$^-$) pointed to a methyl-luteolin as aglycone (Ferreres et al., 2007). All in all, the compound was tentatively assigned as methyl-luteolin 2″-*O*-deoxyhexosyl-*C*-hexoside.

Finally, peak $16^{Cc}$ showed a pseudomolecular ion $[M-H]^-$ at *m/z* 575 releasing fragment ions at *m/z* 411 ([(M-H)-146-$H_2O$]$^-$, base peak) and at *m/z* 429 ([(M-H)-146]$^-$) that suggested the presence of a 2″-*O*-linked deoxyhexosyl moiety (Ferreres et al., 2007). The fragments at *m/z* 367 and 337, from further loss of 44 and 74 mu from the base peak, respectively, indicated a *C*-linked 6-deoxyhexose, and the observation of an unusual fragment at *m/z* 309 from the loss of 102 mu (instead of 104 mu) from the base peak could be interpreted as the existence of a ketone carbon in the sugar residue. All in all, the compound was tentatively identified as luteolin 2″-*O*-deoxyosyl-6-*C*-(6-deoxy-*pento*-hexos-ulosyl) (Fig. 2C), similar to the compound previously reported in *C. citratus* by Figueirinha et al. (2008).

In *C. citratus*, flavonoids were the major group found being luteolin 2″-*O*-deoxyhexosyl-6-*C*-hexoside (peak $6^{Cc}$, 2138.07 μg/g dw) the main compound (Table 3). Figueirinha, Cruz, Francisco, Lopes, and Batista (2010); Figueirinha et al. (2008) presented a very similar profile to the one shown for *C. citratus* in this study. Otherwise, Marques and Farah (2009) only detected the presence of caffeoylquinic, feruloylquinic and

dicaffeoylquinic acid derivatives in methanolic and infusions of *C. citratus* from Brazil. Furthermore, Port's, Chisté, Godoy, and Prado (2013) also studied a sample from Brazil, but they submitted their samples to a hydrolysis process revealing a completely different profile (gallic acid, catechin, epicatechin, quercetin, rutin and myricetin).

### 3.2. Antioxidant activity

Antioxidant activity cannot be measured directly and numerous tests have been developed for measuring the antioxidant capacity of food and biological samples. However, there is no universal method that can measure the antioxidant capacity of all samples accurately and quantitatively (Prior, Wu, & Schaich, 2005). Furthermore, standardized methods for antioxidant activity should meet certain requirements, and therefore the methods of assessing antioxidant capacity fall into two broad categories reflecting the focus on radicals scavenging activity and lipid peroxidation inhibition (Magalhães, Segundo, Reis, & Lima, 2008). In the present study, the antioxidant activity was assessed by DPPH scavenging activity, reducing power and inhibition of lipid peroxidation (β-carotene bleaching inhibition and TBARS assays).

The results of the antioxidant activity of the three studied plant species are presented in Table 4. *P. tridentatum* methanolic extract gave the highest DPPH scavenging activity and reducing power. This might be explained by its peculiar profile in phenolic compounds, mainly dihydroflavonol and isoflavone derivatives. Isoflavones have been extensively studied for their possible health-promoting effects. These phenolic compounds have the potential to scavenge free radicals such as superoxide and nitric oxide (Rimbach et al., 2003). Genistein and daidzein are known to be the most effective isoflavones, possessing direct free radical quenching ability (Arora, Nair, & Strasburg, 1998; Ruiz-Larrea et al., 1997). Furthermore, isoflavones are also known to have the ability to decrease oxidative damage in cells via indirect mechanisms, such as induction of antioxidant-scavenging enzymes (Cai & Wei, 1996). Recently, dihydroflavonol has also received attention due to its potential health benefits, attributed to the antioxidant activity. It has been described that the antioxidant properties of these phenolic compounds are the results of the high propensity to transfer electrons, to chelate ferrous ions and to scavenge reactive oxygen species (Gong et al., 2009; Montoro, Braca, Pizza, & De Tommasi, 2005).

Nevertheless, it was *C. citratus* that showed the highest β-carotene bleaching and lipid peroxidation inhibitions. This could be explained by its higher amount in flavonoids, especially apigenin and luteolin derivates. Moreover this species also revealed a high amount of *C*-glycosylflavones, which have been found to present an antioxidant properties (Talhi & Silva, 2012). Figueirinha et al., 2008 proved that the flavonoid fraction of *C. citratus* (mostly apigenin and luteolin *C*-glycosylflavones derivatives) demonstrated to have a good scavenger capacity for superoxide anion and hydroxyl radical, revealing that these compounds possess a protective effect against those reactive species which are involved in inflammatory and degenerative diseases.

*G. globosa* methanolic extract gave the lowest activity in all the assays, presenting also the lowest phenolic concentrations, which might explain the less effect shown by this sample.

The studied *C. citratus* extract gave higher DPPH scavenging activity than methanolic extracts obtained from a commercial sample from Taiwan (23.5% at 1 mg/mL; Tsai, Tsai, Chien, Lee, & Tsai, 2008) and Malaysia ($EC_{50}$ value 994.77 μg/mL; Koh et al., 2012), and also higher reducing power than an ethanolic extract from a Korean sample (absorbance 0.32 at 0.2 mg/mL; Oh, Jo, Cho, Kim, & Han, 2013). Nonetheless, it gave lower DPPH scavenging activity than a methanolic extract prepared with samples from Chile (67.9% at 33 μg/mL; Cheel et al., 2005) and Brazil (0.08 μg/mL; Port's et al., 2013).

*G. globosa* extract presented a lower DPPH scavenging activity ($EC_{50}$ value 421 μg/mL) when compared to an aqueous extract of a commercial sample of *G. globosa* also from Portugal, but from a different distributor (Silva et al., 2012). Finally, the studied *P. tridentatum* sample gave higher DPPH scavenging activity and reducing power, but lower lipid peroxidation inhibition when compared to the one described by the authors for a wild sample traditionally shade-dried (Pinela et al., 2011).

Overall, *C. citratus* showed the highest β-carotene bleaching and lipid peroxidation inhibitions, that can be due to its high amount in flavonoids, especially apigenin and luteolin derivatives (luteolin 2″-*O*-rhamnosyl-6-*C*-glucoside was the main compound). *P. tridentatum* revealed the highest DPPH radical scavenging activity and reducing power, that may be explained by its peculiar profile in phenolic compounds, mainly dihydroflavonol and isoflavone derivatives. Otherwise, *G. globosa* showed the highest content of kaempferol 3-*O*-rutinoside and betacyanins were only present in this sample.

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**Table 4**
Antioxidant activity ($EC_{50}$ values, mg/mL) of *P. tridentatum*, *G. globosa* and *C. citratus* methanolic extracts (mean ± SD).

| | *Pterospartum tridentatum* | *Gomphrena globosa* | *Cymbopogon citratus* |
|---|---|---|---|
| DPPH scavenging activity | $0.18 \pm 0.01^{c}$ | $4.87 \pm 0.13^{a}$ | $0.31 \pm 0.02^{b}$ |
| Reducing power | $0.11 \pm 0.00^{c}$ | $1.47 \pm 0.03^{a}$ | $0.21 \pm 0.01^{b}$ |
| β-Carotene bleaching inhibition | $0.48 \pm 0.09^{b}$ | $0.61 \pm 0.03^{a}$ | $0.32 \pm 0.10^{c}$ |
| TBARS inhibition | $1.18 \pm 0.06^{b}$ | $3.31 \pm 0.18^{a}$ | $0.53 \pm 0.01^{c}$ |

$EC_{50}$ values correspond to the extract concentration achieving 50% of antioxidant activity or 0.5 of absorbance in reducing power assay. In each row different letters mean significant differences ($p < 0.05$).

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