



Original Article

Antiproliferative activity, antioxidant capacity and chemical composition of extracts from the leaves and stem of *Chresta sphaerocephala*



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ABSTRACT

In this study, antiproliferative and antioxidant activities of crude extracts (hexane, ethyl acetate and methanol) from leaves and stem of *Chresta sphaerocephala* DC., Asteraceae, were investigated. Antiproliferative activity was tested *in vitro* against ten human cancer cells and against VERO (no cancer cell). Antioxidant activities were determined using DPPH and ORAC-FL assays and the total phenolic content was estimated by Folin–Ciocalteu method. Hexane and ethyl acetate extracts (leaves and stem) exhibited antiproliferative activity against cancer cell lines with total growth inhibition (TGI) between 50.40 and 250 $\mu\text{g/ml}$. For VERO cell, TGI values were $>250 \mu\text{g/ml}$ for all extracts, except to hexane extract of the stem (TGI 80.92 $\mu\text{g/ml}$). In an initial evaluation, ethyl acetate and methanol extracts (leaves and stem) have shown levels of phenolic compounds between 6.94 and 30.96 mg GAE/kg in Folin–Ciocalteu assay, DPPH free-radical scavenging activity with SC_{50} in the range of 75.22 and 400 $\mu\text{g/ml}$ and antioxidant capacity between 290.08 and 1088 $\mu\text{mol TE/g}$ of extract in ORAC-FL assay. HPLC-DAD and ESI-MS analysis allowed the identification of flavonoids in the methanol extract from the leaves of *C. sphaerocephala*. Three steroids and nine triterpenoids were identified in the bioactive hexane extracts using HRGC.

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Introduction

Natural products are small molecules that can present bioactive structure by itself or be employed as precursors for the synthesis of different drugs (Kumar et al., 2014). Therefore, they are major source for drug discovery or can help in the comprehension of biochemical pathway related to pathological processes (Rishton, 2008). A great diversity of organisms can be a source of natural products with emphasis on terrestrial plants. Plants are the basis of traditional medicine around the world, in which many drugs used today were discovered. Ethnobotany and ethnopharmacognosy studies assist in the identification of plant species with pharmacological potential, as well as to identify sources of different compound classes (Gurib-Fakim, 2006).

Nonetheless, studies for the development of validated, safe and effective natural products, coupled with sustainable use, are increasingly needed (Farias, 2004). In developing countries, about two thirds of the population uses plants as a source of drugs without any scientific basis, a practice that can lead to acute or chronic poisoning. In Brazil, it is also a reality, further aggravated due to an abundance of plant species unknown, both taxonomically and chemically.

Moreover, Brazil displays a great biodiversity and an intrinsic chemical diversity, which ensures a potential for bioprospecting from its natural resources. Therefore, studies to trace chemical and pharmacological profile of this biological wealth, seen as a promising alternative for the primary health care, are required (Maciel et al., 2002).

The Asteraceae family has many species with ethnopharmacological and medicinal uses, being popularly used for its anti-inflammatory, antiseptic and anti-tumor properties. Phytochemical studies of Asteraceae species report the occurrence of

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Table 1
Antiproliferative activity of *Chresta sphaerocephala* extracts.

Samples	TGI (μg/ml) ^a										
	U251	UACC-62	MCF-7	NCI-ADR/RES	786-0	NCI-H460	PC-03	OVCAR-3	HT-29	K562	VERO
<i>Chresta sphaerocephala</i>											
Extracts of leaves											
HEL	98.6	125.2	>250	210.2	72.3	210.8	135.9	58.3	198.9	152.1	>250
EEL	>250	>250	>250	250.0	>250	>250	>250	172.3	>250	>250	>250
MEL	>250	>250	>250	>250	>250	>250	>250	>250	>250	250.0	>250
Extracts of stem											
HES	77.6	143.9	250.0	87.0	94.6	90.1	250.0	50.4	72.6	250.0	80.9
EES	90.8	>250	>250	>250	>250	>250	>250	199.3	>250	118.6	>250
MES	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
Doxorubicin ^b	6.3	1.2	>25	>25	25	>25	3.5	3.8	>25	1.0	6.9

^a TGI – Total growth inhibition – concentration that inhibited cell growth by 100%. The coefficients of variation obtained in these analyses were below to 5%.

^b Positive control.

Cell lines: U251 (glioma); UACC-62 (melanoma); MCF-7 (breast); NCI-ADR/RES (ovarian-resistant); 786-0 (kidney); NCI-H460 (lung); PC-03 (prostate); OVCAR-3 (ovarian); HT-29 (colon); K562 (leukemia); VERO (normal epithelial renal cell line, green monkey).

triterpenes, sesquiterpene lactones, lignans, flavonoids and caffeoylquinic acid derivatives (Schinor et al., 2006; Gobbo-Neto and Lopes, 2008; Salvador et al., 2009), related to antioxidant, analgesic and antiprotozoal properties.

The genus *Chresta*, comprises twelve endemic species in central Brazil, and some species showed antibacterial and antiprotozoal activities (Schinor et al., 2006, 2007a,b; Robinson, 1999). These species are recognized by the population of northeastern Brazil as traditional herbs used to treat gastric diseases and other disorders, hence presenting ethnopharmacological relevance (Silva et al., 2012).

Chresta sphaerocephala DC., is an herbaceous species from the tribe Vernonieae, Asteraceae, known as “joão-bobo”. It strictly occurs in the region of Brazilian cerrado. Few species of the *Chresta* genus have been studied regarding its chemical composition and pharmacological potential. *C. exsucca* and *C. scapigera* presented significant potential regarding antimicrobial activity against bacteria, fungi and protozoa and steroids, terpenoids and flavonoids were identified on their bioactive extracts (Schinor et al., 2006, 2007a,b). These results indicate that species of this genus can be sources of active compounds. To the best of our knowledge, no reports about cytotoxic and antioxidant properties *C. sphaerocephala* has been documented in the literature. This fact prompted us to investigate these activities of crude extracts (hexane, ethyl acetate and methanol) from the leaves and stem of *C. sphaerocephala*, as well as, explore in more detail its chemical composition by different chromatographic techniques.

Materials and methods

Plant material

Chresta sphaerocephala DC., Asteraceae, was collected in its natural habitat, Furnas-MG, Brazil. The botanical identification was carried out by Prof. Dr. João Semir (Departamento de Biologia vegetal, Instituto de Biologia, Universidade de Campinas) and a voucher specimen has been deposited in the herbarium of Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da Universidade de São Paulo (SPFR 06875).

Plant extracts

After drying the plant material (leaves and stem) in a circulating air oven at 40 °C, it was pulverized in grinder knives and subjected to the process of maceration with organic solvents, hexane, ethyl acetate (EtOAc) and methanol, at a powder/solvent ratio of 1:20 (w/v). After filtration, solvents were removed under reduced

pressure to obtain the crude extracts denominated as follows: hexane extracts from the leaves (HEL) and stem (HES), ethyl acetate extracts from leaves (EEL) and stem (EES) and methanol extracts from leaves (MEL) and stem (MES). These extracts were properly packed and stored.

Antiproliferative assay

Human tumor cell lines U251 (glioma), UACC-62 (melanoma), MCF7 (breast), NCI-ADR/RES (ovarian expressing multiple drug resistance phenotype), 786-0 (kidney), NCI-H460 (lung, non-small cells), PC-3 (prostate), OVCAR-3 (ovary), HT29 (colon) and K-562 (leukemia) were kindly provided by National Cancer Institute at Frederick MA-USA (NCI). Also, a normal cell line (VERO, renal, green monkey) was used. Stock cultures were grown in medium containing 5 ml RPMI 1640 (GIBCO BRL) supplemented with 5% fetal bovine serum. Penicillin:streptomycin mixture (1000 UI/ml:1000 μg/ml, 1 ml/LRPMI 1640) was added to experimental cultures. Cells in 96-well plates (100 μl cells/well) were exposed to different sample concentrations in DMSO/RPMI (0.25, 2.5, 25, and 250 μg/ml) and incubated at 37 °C, 5% of CO₂ in air for 48 h. Final DMSO concentration did not affect cell viability. Afterwards, cells were fixed with 50% trichloroacetic acid and cell proliferation was determined by spectrophotometric quantification (540 nm) of cellular protein content using sulforhodamine B assay. Doxorubicin chloride (0.1 mg/mg; Europharma) was used as a positive control. Three measurements were obtained: at time zero (T_0 , at the beginning of incubation) and 48 h post-incubation for compound-free (C) and tested (T) cells. Cell proliferation was determined according to the equation $100 \times [(T - T_0)/C - T_0]$, for $T_0 < T \leq C$, and $100 \times [(T - T_0)/T_0]$, for $T \leq T_0$ and a concentration–response curve for each cell line was plotted using software Origin 8.0 (OriginLab Corporation). From the concentration–response curve for each cell line, TGI (concentration that produces 100% of cell growth inhibition or cytostatic effect) value was determined through non-linear regression analysis using the software Origin 8.0[®] (OriginLab Corporation) (Monks et al., 1991; Shoemaker, 2006). The results are depicted in Table 1 and Fig. 1.

DPPH radical reduction

The radical DPPH (2,2-diphenyl-1-picrylhydrazyl) is stable, pale purple, and when reduced, turns yellow. In this assay, it was evaluated standards and samples ability to reduce DPPH radical. Briefly, 1 mg of each extract was dissolved in methanol (10 ml), yielding a stock solution. Several dilutions were prepared (20–200 μg/ml) in methanol and for each sample (1 ml) was added 2 ml of DPPH

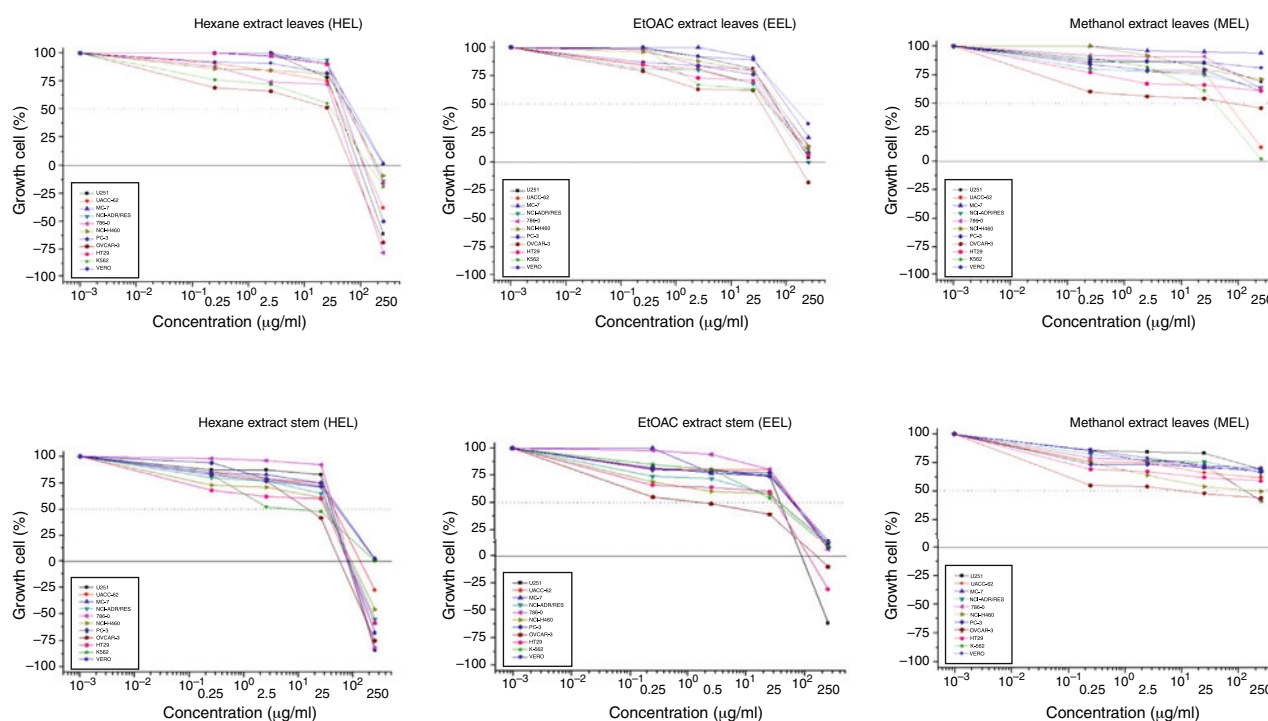


Fig. 1. Antiproliferative activity of *Chresta sphaerocephala* extracts.

(10 mg/ml). After 30 min, the absorbance was measured by a spectrophotometer ($\lambda = 517$ nm) and the percentage of anti-radical activity was calculated (Huang et al., 2005). As a positive control it was used quercetin and trolox and the dilution solution (methanol) as negative control. Antioxidant activity of each sample was expressed as the SC_{50} value, which is the concentration in $\mu\text{g/ml}$ of each extract that scavenged 50% of DPPH radical. All experiments were performed in triplicate (Table 2).

Evaluation of antioxidant capacity by ORAC assay

Antioxidant activity of extracts was measured applying ORAC-FL assay, with fluorescein as fluorescent probe and AAPH [2,2'-azobis (2-amidopropane) dihydrochloride] as a source of radical peroxy. The experiments were performed in 96 wells microtiter plates as described by Ou et al. (2001) and Prior et al.

(2003) with modifications (Salvador et al., 2006). For this reason, extracts (50 mg/ml) and fractions (5 mg/ml) stock solutions were prepared in phosphate buffer/DMSO (99:1) and diluted 100, 500, 1000, 5000 and 10,000 times with phosphate buffer. Both compounds and trolox (reference substance, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid – vitamin E analog) were analyzed at concentrations of 6.25, 12.5, 25, 50, 100 and 200 μM , using the same solvent system. The reading was performed using fluorescent filter (excitation $\lambda = 485$ nm and emission $\lambda = 528$ nm) in a microplate reader by monitoring their reaction kinetics every 2 min for a period of 70 min, at 37 °C. Results were expressed as μmol of trolox equivalent (TE) per gram of extract in dry basis ($\mu\text{mol TE/g}$) and as trolox equivalent relative to isolated compounds (positive controls). Quercetin, isoquercitrin, caffeic acid and chlorogenic acid were used as positive controls and the dilution solution as negative control. All experiments were performed in triplicate (Table 2).

Table 2

Total phenol content and antioxidant capacity by the DPPH and ORAC assays of *Chresta sphaerocephala* extracts.

Samples	Phenol content ^a (mg of GAE/kg) ^b	DPPH assay, SC_{50} ^a ($\mu\text{g/ml}$) ^c	ORAC ^a ($\mu\text{mol TE/g}$) ^d
Hexane extract leaves (HEL)	<5.0	–	–
EtOAc extract leaves (EEL)	30.9 (0.07)	>400	290.1 (0.23) ^d
Methanol extract leaves (MEL)	14.6 (0.05)	75.22 (3.00)	1088 (0.12) ^d
Hexane extract stem (HES)	<5.00	–	–
EtOAc extract stem (EES)	6.94 (0.06)	>400	<200 ^d
Methanol extract stem (MES)	17.24 (0.04)	400 (6.80)	471.2 (0.17) ^d
Quercetin [*]	–	12.40 (2.00)	5.60 (0.75) ^e
Isoquercitrin [*]	–	–	5.15 (1.20) ^e
Caffeic acid [*]	–	–	2.98 (1.90) ^e
Chlorogenic acid [*]	–	–	2.40 (1.70) ^e
Trolox [*]	–	9.60 (1.80)	–

^{*} Experimental positive controls.

– Not evaluated.

^a Mean (%RSD, relative standard deviation) of triplicate assays.

^b Total phenolics data expressed as milligrams of gallic acid equivalents per gram (mg of GAE/kg) of extract.

^c DPPH assay data expressed as SC_{50} (concentration that inhibited 50% of the DPPH radical) in micrograms per milliliters ($\mu\text{g/ml}$).

^d ORAC data expressed as micromol of Trolox equivalents per gram ($\mu\text{mol of TE/g}$) of extract.

^e ORAC data expressed as relative Trolox equivalent, mean (%RSD, relative standard deviation) of triplicate assays.

TLC autographic assay for DPPH radical-scavenging and EASI-MS analysis of antioxidant extracts

TLC autographic chromatography assay was performed with antioxidant methanol extract from leaves of *C. sphaerocephala* and TLC chromatography yellow spot with antioxidant activity were analyzed by EASI-MS (easy ambient sonic-spray ionization) via ambient desorption/ionization and (tandem) mass spectrometry detection (Haddad et al., 2008). Thus, 10 µl of extract solution (1 mg/ml) in methanol was applied into two TLC plates (silica gel 60 GF254, Fluka, AG, Switzerland, *n*-butanol/acetic acid/water, 65:15:25, v/v). One of the TLC plate was sprayed with a 0.2% 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma–Aldrich, St Louis, MO) solution in MeOH and left at room temperature and the other plate (control, without DPPH spraying) was analyzed only by EASI-MS. Plates were observed 30 min after spraying. Active compounds are observed as yellow spots against a purple background. As both TLC were developed together, the yellow spots in first TLC (sprayed with DPPH) had served as a guide to choose areas in the other TLC plate that would be analyzed by EASI-MS according to Haddad et al. (2008) and MS or MS/MS characterization were performed. All experiments were performed in triplicate.

Quantitative determination of total soluble phenols

Extracts, dissolved in methanol, were analyzed for their total soluble phenolic content according to Folin–Ciocalteu colorimetric method (Piccinelli et al., 2004; Wu et al., 2004), using gallic acid as reference (FCR assay). Results were expressed as milligrams of gallic acid equivalents (GAE) per gram of extract (mg of GAE/g). Analyses were performed in triplicate (Table 2).

Clean up procedure

The experiments were performed as described by Schinor et al. (2004). An aliquot (10 mg) of HEL and HES was resuspended in analytical grade chloroform (3 ml) and percolated through a sep-kap column (Alltech, silica-gel 200 mg, 3 ml). The column was eluted with hexane (10 ml) and chloroform (10 ml). Fractions were collected separately and evaporated to dryness at room temperature. Chloroform phase was analyzed by HRGC below. MEL and MES extracts (10 mg) were resuspended in methanol–water (7:3, v/v, 3 ml) and percolated through a sep-kap column (Alltech, C-18, 200 mg, 3 ml). The column was eluted with analytical grade methanol (10 ml) and this phase was analyzed by HPLC–ESI-MS.

Gas chromatographic analysis

Chloroform phase was analyzed by HRGC on a Hewlett-Packard model 5890 Series II Gas Chromatograph with a split injector (split ratio 1:60, v/v) at 260 °C and a flame ionization detector at 330 °C. The injected volume was 2 µl. Hydrogen was employed as carrier gas at an average linear velocity of 44 cm/s (HP-50) and 42 cm/s (HP-1). An HP-50 (cross-linked 50% phenyl-methylsilicone, 30 m × 0.25 mm × 0.25 mm) and an HP-1 (cross-linked methyl-silicone, 30 m × 0.25 mm × 0.25 mm) capillary columns were employed. For HP-50 the column temperature was 280 °C (isotherm) and for HP-1, the column temperature program was 250 °C held for 12 min, increased at 6 °C/min to 280 °C, and held this temperature for 30 min. Each sample was analyzed in duplicate. Data were processed on a Hewlett-Packard model 3395 injector. The standards used in the gas chromatography are listed in Table 3.

Table 3

Chemical composition of hexane extracts (HEL and HES) of *Chrestasphaerocephala* (steroids, triterpenoids and triterpenoid acetates) analyzed by gas chromatography (HP-50 column).

Compounds	Leaves	Stem	RR ^a
Campesterol	–	+	1.236
Stigmasterol	+	+	1.306
β-Sitosterol	–	+	1.450
Stigmast-7en-3β-ol	+	+	1.509
Spinasterol	–	–	1.676
Taraxerone	–	–	1.628
Epitaraxerol	–	–	1.656
Taraxerol	–	–	1.698
β-Amyrin	+	+	1.760
α-Amyrin	+	+	1.983
Lupeol	+	+	2.041
β-Friedelanol	–	+	2.476
Friedelin	–	+	2.709
Pseudotaraxasterol	–	–	2.467
Taraxasterol	–	+	2.561
11-Oxours-12-ene	–	–	3.047
11-Oxolean-12-ene	–	–	3.386
Taraxerolacetate	–	–	1.866
β-Amyrinacetate	+	+	1.923
α-Amyrinacetate	+	+	2.145
Lupeolacetate	+	+	2.224
Bauerenylacetate	–	–	2.497
11α-12α-Oxidetaraxeryl	–	–	2.795
β-Friedelanolacetate	–	–	2.857
α-Amyrinononilacetate	–	–	3.637
β-Amyrinononilacetate	–	–	4.107

– Compound not detected.

+ Compound detected.

^a Relative retention time related to the internal standard (cholesterol) in minutes.

HPLC–DAD

Methanol extracts, MEL and MES, were investigated by HPLC and the analysis was carried out with a Waters Alliance equipment, using a C-18 Polaris–Varian analytical column (4.6 mm × 250 mm). The mobile phase consisted of a linear gradient combining solvent A (methanol) and solvent B (water/formic acid, 99:1, v/v) as follows: 15% A (0 min), 15–90% A (30 min), 90% A (5 min), 90–15% A (5 min). The analyses were performed in triplicate at a flow rate of 1.0 ml/min with the UV detector set at λ = 280 nm and an injection volume of 10 µl. Subsequent samples were injected with an interval of 10 min after the previous sample run.

HPLC–ESI-MS analysis

The ESI-MS spectra were acquired on a Q-trap (Applied Biosystems) Mass Spectrometer with an ESI source operated in the negative ion mode. The source and desolvation temperatures were 70 °C and 100 °C, respectively. Cone voltage was 30 V. The parent ion and the retention time were compared with previously isolated flavonoids (Table 4) of our research group, as described by Schinor et al. (2006, 2007a,b).

Statistical analysis

Data are reported as mean (%RSD, relative standard deviation) and analyzed by ANOVA the Tukey test, *p*-value <0.05.

Results and discussion

The antiproliferative activity of the extracts of *C. sphaerocephala* (leaves and stem) was investigated on a series of cancer cell lines by sulforhodamine B assay. The results showed antiproliferative effect against all cell lines only for hexane extracts with total growth inhibition (TGI) values between 50.4 and 250 µg/ml (Table 1, Fig. 1).

Table 4Flavonoids identified by HPLC and ESI-MS on methanol extracts (MEL and MES) of *Chrestasphaerocephala*.

Compounds	Rt. (min) ^a	[M-H] ⁻ (m/z) ^b	Present flavonoids in methanol extract	
			Leaves	Stem
Apigenin	24.56	269	+	–
Luteolin	22.72	285	+	–
Genkwanin	29.20	283	+	–
Crysoeriol	24.78	299	+	–
Velutin	29.01	313	+	+
Luteolin-3'-O-β-D-glucopyranoside	19.38	447	+	–
Luteolin-7'-O-β-D-glucopyranoside	11.08	447	+	–
Vicenin-2	12.63	593	+	–

+ Compound detected.

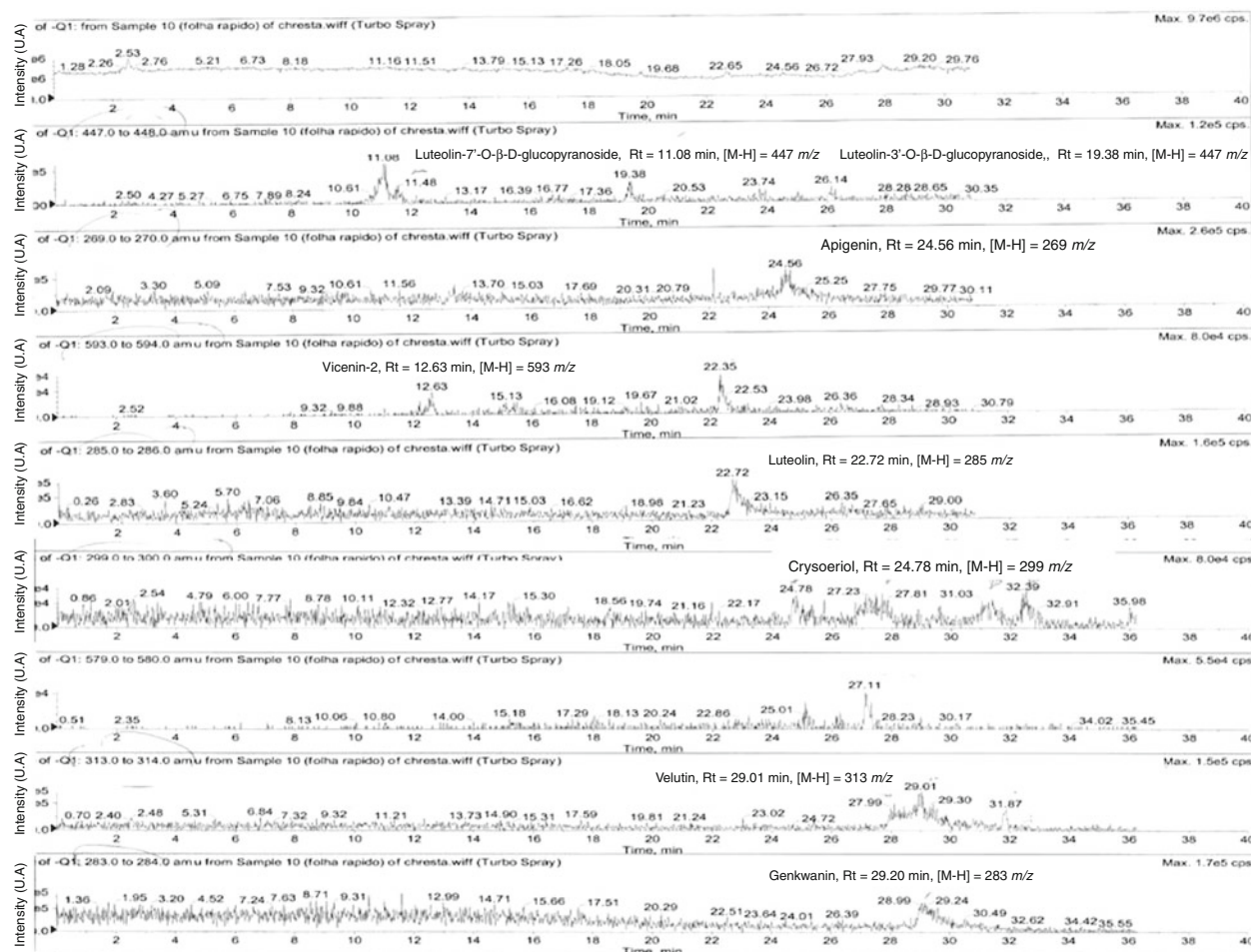
– Compound not detected.

^a Values of retention time.^b ESI-MS analysis.

For both HES and HEL extracts, the cell line OVCAR-3 (ovarian cancer cell line) was more susceptible with TGI values equal to 50.4 and 58.3 μg/ml, respectively. HES also showed weak cytostatic effect against HT29 (colon, TGI = 72.6 μg/ml), U251 (glioma, TGI = 77.6 μg/ml), NCI-ADR/RES (ovarian expressing multiple drug resistance phenotype, TGI = 87.0 μg/ml), NCI-H460 (lung, non-small cells, TGI = 90.1 μg/ml) and 786-0 (renal, TGI = 94.6 μg/ml). This extract also inhibited cell growth of VERO cells (renal, green monkey) at the same level that affected cancer cells (TGI 80.9 μg/ml). In general, both methanol and ethyl acetate extracts were inactive against all cell lines. These results indicate that the

antiproliferative activity of *C. sphaerocephala* are probably due to nonpolar compounds.

In FCR assay, both methanol and ethyl acetate extracts, presented significant levels of phenolic compounds (Table 2). The amount of gallic acid in MEL (14.6 mg/kg) and MES were similar (17.2 mg/kg), while this compound content at EEL (30.9 mg GAL/g) were almost five times higher than the one found in EES (6.9 mg GAE/kg). Only MEL showed expressive DPPH scavenge ability with SC₅₀ values equal to 75.2 μg/ml whereas for EEL, EES and MES, SC₅₀ were higher than 400 μg/ml. On the other hand, at ORAC-FL assay, these extracts demonstrated antioxidant capacity between

**Fig. 2.** Representative chromatogram of UPLC-MS of methanol extract from the leaves of *Chresta sphaerocephala*.

290.1 and 1088.1 $\mu\text{mol TE/g}$ of extract, except for EES, which did not reach measurable concentrations according to experimental procedure. Hexane extracts (HEL and HES) showed no antioxidant activity in both tests. Therefore, it is possible to assert that DPPH radical reduction and antioxidant capacity of methanol and ethyl acetate extracts are in good correlation with the total phenolic content measured by Folin–Ciocalteu, once these classes of compounds are known as potent antioxidants.

In chemical profile characterization of HES and HEL by GC analysis, allowed the identification of three steroids (stigmasterol, β -sitosterol and stigmast-7-en- β -ol) and nine triterpenoids (α -amyrin, β -amyrin, lupeol, taraxasterol, friedelin, friedelanol, α -amyrin acetate, β -amyrin acetate and lupeol acetate) as shown in Table 3. HPLC-DAD and ESI-MS analyses revealed that both methanol extracts present flavonoids as major constituents, such as luteolin-3-O- β -D-glucopyranoside, luteolin-7-O- β -D-glucopyranoside, vicenin-2, luteolin, crysoeriol, velutin, apigenin and genkwanin (Table 4; Fig. 2). TLC chromatography analysis of MEL extract (the one with higher antioxidant activity), followed by EASI-MS analysis of the yellow spot revealed in the TLC plate for antioxidant activity showed the substance as being apigenin. In this case, not only, these extract (MEL) antioxidant activity is probably attributed to these flavonoid but also, the combination of both analytical techniques provided a simple and advantageous technique for the separation and detection of an active compound.

Moreover, the compounds stigmasterol and lupeol found in hexane extracts have already been described as possessing promising antiproliferative activities and could be related to the results observed in this study (Yasukawa et al., 1991; Kasahara et al., 1994; Saleem, 2009). On the other hand, luteolin and apigenin, observed in methanol extracts, are reported as outstanding antioxidant/free-radical scavenging substances (Burda and Oleszek, 2001; Romanova et al., 2001; Sala et al., 2003; Shohreh et al., 2008).

Despite the promising results found here, further biological studies should be performed, including *in vivo* investigations, looking toward a clinical employment of these bioactive natural products.

Author contributions

LSC and NLA carried out all experimental procedures of this study, data analysis and interpretation and drafted the manuscript. WRC assisted in antioxidant assays. ALTGR and JEC assisted in the antiproliferative biological assays. IBSC carried out ESI-MS analysis and assisted in spectra data interpretation. ECS, DAD and MJS conceived the study and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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References

Burda, S., Oleszek, W., 2001. Antioxidant and antiradical activities of flavonoids. *J. Agric. Food Chem.* 49, 2774–2779.

- Farias, M.R., 2004. Avaliação da qualidade de materias-primas vegetais. In: Simões, C.M.O., Schenkel, E.P., Gosmann, G., Mello, J.C.P., Mentz, L.A., Petrovick, P.R. (Eds.), *Farmacognosia: da planta ao medicamento*, 5th ed. Editora da Universidade UFRGS/Editora da UFSC, Porto Alegre/Florianópolis, pp. 263–288.
- Gobbo-Neto, L., Lopes, N.P., 2008. Online identification of chlorogenic acids, sesquiterpene lactones, and flavonoids in the Brazilian amaranth *Lychnophora ericoides* Mart (Asteraceae) leaves by HPLC-DAD-MS and HPLC-DAD-MS/MS and a validated HPLC-DAD method for their simultaneous analysis. *J. Agric. Food Chem.* 56, 1193–1204.
- Gurib-Fakim, A., 2006. Medicinal plants: traditions of yesterday and drugs of tomorrow. *Mol. Asp. Med.* 27, 1–93.
- Haddad, R., Milagre, H.M.S., Catharino, R.R., Eberlin, M.N., 2008. Easy ambient sonic-spray ionization mass spectrometry combined with thin-layer chromatography. *Anal. Chem.* 80, 2744–2750.
- Huang, D., Ou, B., Prior, R.L., 2005. The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* 53, 1841–1856.
- Kasahara, Y., Kumaki, K., Katagiri, S., Yasukawa, K., Yamanouchi, S., Takido, M., Akihisa, T., Tamuta, T., 1994. *Carthamiiflos* extract and its component, stigmasterol, inhibit tumour promotion in mouse skin two-stage carcinogenesis. *Phytother. Res.* 8, 327–331.
- Kumar, S.V., Saravanan, D., Kumar, B., Jayakumar, A., 2014. An update on prodrugs from natural products. *Asian Pac. J. Trop. Med.* 7, S54–S59.
- Maciel, M.A.M., Pinto, A.A., Veiga, V.F.J., 2002. Plantas medicinais: a necessidade de estudos multidisciplinares. *Quim. Nova* 25, 429–438.
- Monks, A., Scudiero, D., Skehan, P., Shoemaker, R., Paull, K., Vistica, D., Hose, C., Langley, J., Cronise, P., Vaigro-Wolff, A., Gray-Goodrich, M., Campbell, H., Mayo, J., Boyd, M., 1991. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J. Natl. Cancer Inst.* 83, 757–766.
- Ou, B., Hampsch-Woodill, M., Prior, R.L., 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J. Agric. Food Chem.* 49, 4619–4626.
- Piccinelli, A.L., De Simone, F., Passi, S., Rastrelli, L., 2004. Phenolic constituents and antioxidant activity of *Wendita calysina* leaves (burrito), a folk Paraguayan tea. *J. Agric. Food Chem.* 52, 5863–5868.
- Prior, R.L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., Hampsch-Woodill, M., Huang, D., Ou, B., Jacob, R., 2003. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC-FL)) of plasma and other biological and food samples. *J. Agric. Food Chem.* 51, 3273–3279.
- Rishton, G.M., 2008. Natural products as a robust source of new drugs and drug leads: past successes and present day issues. *Am. J. Cardiol.* 101, S43–S49.
- Robinson, H., 1999. In: Smithsonian Institution Press (Ed.), *Generic and Subtribal Classification of American Vernoniae*. Smithsonian Contributions to Botany, Washington, DC, pp. 9–12.
- Romanova, D., Vacakova, A., Cipak, L., Ovesna, Z., Rauko, P., 2001. Study of antioxidant effect of apigenin, luteolin and quercetin by DNA protective method. *Neoplasma* 48, 104–107.
- Sala, A., Recio, M.C., Schinella, G.R., Mañez, S., Giner, R.M., Cerdá-Nicolas, M., Rios, J.L., 2003. Assessment of the anti-inflammatory activity and free radical scavenger activity of tiliroside. *Eur. J. Pharmacol.* 461, 153–161.
- Saleem, M., 2009. Lupeol, a novel anti-inflammatory and anti-cancer dietary triterpene. *Cancer Lett.* 285, 109–115.
- Salvador, M.J., Ferreira, E.O., Mertens-Talcott, S.U., Castro, W.V., Butterweck, V., Derendorf, H., Dias, D.A., 2006. Isolation and HPLC quantitative analysis of antioxidant flavonoids from *Alternanthera tenella* Colla. *Z. Naturforsch. C* 61, 19–25.
- Salvador, M.J., Sartori, F.T., Sacilotto, A.C.B.C., Pral, E.M., Alfieri, S.C., Vichniewski, W., 2009. Bioactivity of flavonoids isolated from *Lychnophora markgravii* against *Leishmania amazonensis* amastigotes. *Z. Naturforsch. C* 64, 509.
- Schinor, E.C., Salvador, M.J., Turatti, I.C., Zucchi, O.L.A.D., Dias, D.A., 2004. Comparison of classical and ultrasound-assisted extractions of steroids and triterpenoids from three *Chresta* spp. *Ultrason. Sonochem.* 11, 415–421.
- Schinor, E.C., Salvador, M.J., Tomaz, J.C., Pral, E.M.F., Alfieri, S.C., Albuquerque, S., Dias, D.A., 2006. Biological activities and chemical composition of crude extracts from *Chresta exsucca*. *Braz. J. Pharm. Sci.* 42, 83–90.
- Schinor, E.C., Salvador, M.J., Pral, E.M.F., Alfieri, S.C., Albuquerque, S., Dias, D.A., 2007a. Effect of extracts and isolated compounds from *Chresta scapigera* on viability of *Leishmania amazonensis* and *Trypanosoma cruzi*. *Braz. J. Pharm. Sci.* 43, 295–300.
- Schinor, E.C., Salvador, M.J., Ito, I.Y., Dias, D.A., 2007b. Evaluation of the antimicrobial activity of crude extracts and isolated constituents from *Chresta scapigera*. *Braz. J. Microbiol.* 38, 145–149.
- Shoemaker, R.H., 2006. The NCI60 human tumour cell line anticancer drug screen. *Nat. Rev. Cancer* 6, 813–823.
- Shohreh, N., Mehrdad, H., Mehdi, R., Heidari, A.T., 2008. DNA adducts with antioxidant flavonoids: morin, apigenin, and naringin. *DNA Cell Biol.* 27, 433–442.
- Silva, A.A.R., Bezerra, M.M., Chaves, H.V., Pinto, V.P.T., Franco, E.S., Vieira, A.M., Araújo, E.B., Cunha, L.R., Leite, A.C.R., Maia, M.B.S., 2012. Protective effect of *Chresta martii* extract on ethanol-induced gastropathy depends on alpha-2 adrenoceptors pathways but not on nitric oxide, prostaglandins or opioids. *J. Ethnopharmacol.* 142, 206–212.
- Wu, X., Beecher, G.R., Holden, J.M., Haytowitz, D.B., Gebhardt, S.E., Prior, R.L., 2004. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J. Agric. Food Chem.* 52, 4026–4037.
- Yasukawa, K., Takido, M., Matsumoto, T., Takeuchi, M., Nakagawa, S., 1991. Sterol and triterpene derivatives from plants inhibit the effects of tumour promoter and sitosterol and betulinic acid inhibit tumour in mouse skin two-stage carcinogenesis. *Oncology* 41, 72–76.