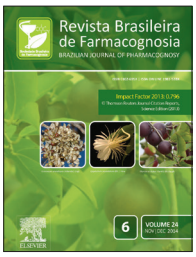
Revista Brasileira de Farmacognosia
BRAZILIAN JOURNAL OF PHARMACOGNOSY
www.journals.elsevier.com/revista-brasileira-de-farmacognosia



Original article

Distribution of metabolites in galled and non-galled leaves of *Clusia lanceolata* and its antioxidant activity

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ARTICLE INFO

Article history:
Received 16 September 2014
Accepted 28 October 2014

Keywords:
Clusia lanceolata
Galls
Essential oil
Phenols
Flavones
Antioxidants

ABSTRACT

Essential oils of galled and non-galled leaves of *Clusia lanceolata* Cambess., Clusiaceae, were obtained by hydrodistillation and analyzed by GC and GC/MS. The chemical composition of both oils was similar, with a predominance of sesquiterpene caryophyllenes. The extracts from the leaves were evaluated regarding total phenols, flavonoids and proanthocyanidins. Galled leaves showed higher levels of phenolics and proanthocyanidins, since the content of flavonoids was higher in non-galled leaves. The chromatographic profiles of extracts were obtained by using HPLC/DAD and LC-ESI-MS. Electrospray ionisation (ESI) in positive and negative ion mode was used to identify four flavones C-glycosides in both extracts. The study constitutes a first report on the chemical research of *C. lanceolata*. The extract from galled leaves had a higher antioxidant activity.

Introduction

Galls are the result of histological alterations in plant organs, mainly hypertrophy and hyperplasy, caused by the activity of an inductor, which can be a virus, bacteria, nematode, insect or other plants (Damasceno et al., 2010). Morphological alterations detected in several plant species due to gall induction are commonly followed by fundamental chemical changes to establish and maintain the host plant-gall maker system. Usually, galls have a high content of nutrients, carbon and energy, in addition to an accumulation of substances via secondary metabolism (Formiga et al., 2009). The mechanisms of host plant resistance in response to insect infestation consist of a series of biochemical events, including increased production of phenolic compounds (Ananthakrishan et al., 1992). The role of some phenolic compounds in plant defence against herbivores and pathogens has been documented in a wide variety of species (Soares et al., 2000; Fields and Orians, 2006). Their specific defensive action depends on the particular compound, but their modes of action include toxicity, growth inhibition, and reduction of digestibility (Barbehenn and Constabel, 2011). Volatile compounds from plants can act as chemical signals for herbivores, and their biosynthesis can be changed as a response to herbivory (Banchio et al., 2005; Damasceno et al., 2008; Torres-Gurrola et al., 2011). Similarly, some of these metabolites can also act as signals to insects for plant host finding and recognition, or as attractants to natural enemies (Tooker and Hanks, 2004; James, 2005).

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0102-695X/$ - see front matter
http://dx.doi.org/10.1016/j.bjp.2014.11.005

The genus *Clusia* comprises about 250 species that occur in tropical and subtropical regions of South and Central America. The species of this genus produce a large amount of latex and floral resins rich in polyprenylated benzophenones (Porto et al., 2000). In addition, xanthones, biflavonoids and triterpenoids have been found in these species (Ferreira et al., 2012; Oliveira et al., 2012). Several species are used in folk medicine worldwide to treat rheumatism, stomach problems and as purgative (Salama, 1986). Plants belonging to this genus are known for their many biological activities, such as antibacterial, antioxidant, antitumor, antimicrobial and anti-inflammatory activities (Gustafson et al., 1992; Iinuma et al., 1996; Cruz and Texeira, 2004). Species of *Clusia* are targets of galls in leaves by herbivory (Santos-Mendonça et al., 2007; Constantino et al., 2009; Fernandes et al., 2010). The insect inducing galls *Clusiamyia nitida* (Diptera, Cecidomyiidae) often infests the shrub *Clusia lanceolata* (Clusiaceae) in the Neotropical sand dunes vegetation in Rio de Janeiro State (Constantino et al., 2009). Studies of this insect-plant interaction have been restricted to investigate the impact of galls on the morphological and physiological aspects of this species. Thus, this study aims to assess the possible impact of the occurrence of galls in the production of volatile and phenolic compounds, especially flavonoids and tannins, in the leaves of *C. lanceolata,* as well as evaluating the antioxidant potential of extracts from galled and non-galled leaves of this species.

Materials and methods

Chemicals materials

The methanol, chloroform and formic acid used for the analysis were of HPLC grade. Follin-Denis reagent, 2,2-diphenyl-1-picryl-hidrazil (DPPH), linoleic acid, ascorbic acid, catechin, quercetin, β-carotene and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma (St. Louis, MO, USA). Vanillin and aluminium trichloride were purchased from Vetec (Brazil). Tween 20 was purchased from Merck (Germany).

Plant material

Leaves of *Clusia lanceolata* Cambess., Clusiaceae, were collected in September 2012 at restinga of Barra de Maricá, Rio de Janeiro State, Brazil. A voucher sample was deposited at the Herbarium of the Universidade Federal Rural do Rio de Janeiro (RBR) under the code 35424.

Isolation of the essential oil and Gas Chromatography analysis (GC-FID and GC-MS)

The essential oils of separated galled and non-galled leaves of *C. lanceolata* Cambess (100 and 200 g, respectively) were obtained by hydrodistillation, using a modified Clevenger apparatus for 2 h. The obtained oil was dried with anhydrous sodium sulphate and stored at 4°C prior to use.

The GC-FID analysis was carried out on a HP5890-Series II apparatus (Agilent Technologies, California, USA) equipped with a flame ionisation detector (FID) and a VF-5 MS Varian fused silica capillary column (30 m x 0.25 mm i.d., 0.25 µm). The oven temperatures were programmed isothermally at 60°C for 2 min, raised to 110°C at a rate of 10°C min^{-1}, then increased to 180°C at a rate of 1.5°C min^{-1}, raised to 240°C at a rate of 10°C min^{-1} and isothermally at 240°C for 10 min. Injector and detector temperatures were 220°C and 250°C, respectively. Helium was used as carrier gas, with a flow rate of 1.0 ml/min^{-1}, and split mode (1:30). Aliquots of 1.0 µl of essential oil were injected, and the percentages of the constituents were calculated by electronic integration of the FID peak areas. GC/MS analyses were performed using a GC/MS-QP 2010 Plus mass spectrometer (Shimadzu, Kyoto, Japan) with a mass selective detector, in EI 70 eV with a scan interval of 0.5 s and fragments from 40 to 550 Da. GC was carried out under the same conditions as described above. Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkanes (Van del Dool and Kratz, 1963), and by comparison of their mass spectral fragmentation patterns with those reported in the literature (Adams, 2007).

Preparation of extracts and chemical analysis

Preparation of extracts

The methanolic extracts of dried galled and non-galled leaves of *C. lanceolata* (20 g) were prepared using a Soxhlet apparatus (10 h). The extracts were filtered using filter paper and then concentrated using a rotary evaporator.

Determination of total phenolic content

The modified Folin-Dennis method (Singleton et al., 1999) was used to determine the total phenolic content. Briefly, 0.5 ml of the extract (0.5 mg ml^{-1} in methanol) was mixed with 2.5 ml of Folin-Denis reagent, and after 5 min, 2 ml of a 14% sodium carbonate (Na_2CO_3) solution was added. After incubation at room temperature for 2 h, the absorbance of the reaction mixture was measured at 760 nm against a methanol blank using a Shimadzu spectrophotometer (UV mini-1240, Japan). A standard curve was constructed using gallic acid (0-0.022 mg ml^{-1}) as reference substance. The mean of three analyses was used, and the total phenolic content was expressed in gallic acid equivalents (mg GAE g^{-1} of extract).

Determination of total flavonoid

Total flavonoid content was determined using a colorimetric method as previously described (Meda et al., 2005). Briefly, 3 ml of a 2% $AlCl_3$ methanolic solution were added to the same volume of extract (0.25 mg m^{-1} in methanol) solution. After 30 min of incubation, the absorbance values were measured at 415 nm against a methanol blank. A standard curve was constructed using quercetin (0-0.020 mg ml^{-1}) as reference substance. The total flavonoid content was calculated from the mean of three analyses and expressed as quercetin equivalents (mg QE g^{-1} of extract).

Determination of condensed tannins

Tannins were determined as described by Price et al. (1978) with some modifications. The tannins were estimated by using 1 ml of extract (1.0 mg ml^{-1} in methanol) solution and 5 ml of a

vanillin/HCl mixture (by mixing equal volumes of 2% vanillin in methanol and 8% methanol/HCl) in a test tube and kept for 20 min at room temperature. The formed colour was determined at 500 nm. A standard curve was constructed using catechin (0-0.040 mg ml^{-1}) as reference substance. The condensed tannins content was calculated from the mean of three analyses and expressed as catechin equivalents (mg CE g^{-1} of extract).

Qualitative HPLC-DAD and LC-ESI-MS/MS analysis

Qualitative analysis of polyphenolswere carried out using a liquid chromatography (LC-20AT, Shimadzu, Japan) with a diode array detector (DAD, SPD-M20A) coupled to an LCSolution ChemStation data-processing station. The column used was a Phenomenex C-18 Luna (150 cm × 4.6 mm; 5 µm), operated at 35°C. The mobile phase consisted of solvent A (water and formic acid, 95:5, pH 2.0) and solvent B (methanol). Elution was performed at a flow rate of 1 ml min^{-1} using a gradient program from 10 to 100% B in 35 min, and isocratic for 5 min. The injection volume was 25 µl and the chromatograms were recorded at 350 nm. Samples and mobile phases were filtered through a 0.45 µm Millipore filter prior to HPLC injection. The LC-ESI-MS/MS was obtained in positive and negative ion mode using an Esquire 3000 Plus-Bruker Daltonics (Bruker Corp., USA) with the conditions: 4000V capillary, 27 psi nebulizer, 7 l/min dry gas and 320 °C dry temp.

Antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

The scavenging activity of extracts for DPPH, was measured as described by Zhang and Hamauzu (2004) with some modifications. A volume of 29 µl of a 0.3 mmol l^{-1} DPPH methanolic solution was added to 71 µl of various concentrations of the extracts (1.5 to 85 µg ml^{-1} in methanol) or the ascorbic acid standard (0.5 at 4 µg ml^{-1} in methanol). The mixtures were kept in the dark for 30 min at room temperature, and the absorbance of the remaining DPPH was determined at 517 nm using a microplate reader (Mensor et al., 2001). A mixture of 29 µl of extract or standard solution was used as a blank, and a mixture of 29 µl of DPPH solution with 71 µl of methanol was used as a negative control. The radical scavenging activity was calculated as percentage of DPPH discoloration using equation (1):

$$AA\ \% = (Asample - Ablank / Acontrol) \times 100 \quad (1)$$

where AA% = inhibition percentage, Abs_{blank} = average absorption of a blank sample at the end of the reaction, Abs_{sample} = average absorption of a extract or standard at the end of the reaction, and $Abs_{control}$ = average absorption of negative control at the end of the reaction. The effective concentration providing 50% inhibition (EC_{50}) was calculated from the graph of scavenging effect percentage against extract or ascorbic acid standard concentration. All experiments were carried out in triplicate.

β-Carotene-linoleic acid assay

The antioxidant activity of extracts on the β-carotene-linoleic acid assay was measured as described by Luís et al. (2009) with some modifications. Briefly, 20 µl of β-carotene solution (20 mg ml^{-1} in chloroform) was added to 40 µl of linoleic acid and 400 mg of Tween 20. This mixture was immediately diluted with 100 ml of distilled water saturated with oxygen. The water was added slowly to the mixture and vigorously agitated to form an emulsion. About 5 ml of the emulsion was transferred into test tubes containing 300 µl of the extracts (1.0 mg ml^{-1} in methanol). About 5 ml of the emulsion and 300 µl of methanol were used as control. Standard Trolox (0.1 mg ml^{-1} in methanol) was used as a reference. The tubes were then gently shaken and placed at 50°C in a water bath for 2 h. The absorbance of the extracts, standard and control were measured at 470 nm, using a spectrophotometer against a blank consisting of an emulsion without β-carotenes. The measurements were carried out at initial time (t=0) and at final time (t=2). All samples were assayed in triplicate. The antioxidant activity was measured in terms of percentage of inhibition of beta-carotene oxidation by:

$$\%\ Inhibition = (Abs^{t=2}sample - Abs^{t=2}control) / (Abs^{t=0}control - Abs^{t=2}control) \times 100$$

where $Abs^{t=2}$ was the absorbance of the sample or control at final time of incubation and $Abs^{t=0}$ was the absorbance in the control at initial time of incubation.

Statistical analysis

Student t-test was performed as a nonparametric test to evaluate the significance of the differences between groups, and $p < 0.05$ was accepted as significant. All statistical analyses were performed with GraphPad Prism 5 DEMO.

Results and discussion

Chemical composition of Clusia lanceolata leaf oil

Upon GC and GC-MS analysis, a total of 28 and 26 components were identified in the essential oil of galled and non-galled leaves of *Clusia lanceolata* Cambess., Clusiaceae, totalling 97.6 and 99.1% of the chemical composition, respectively. Table 1 shows the composition of essential oils extracted from galled (sample A) and non-galled (sample B) leaves. Both oils showed similar composition, except for caryophyllene oxide (6.82% and 0.48%, 14 times higher in sample A), humulene 1,2-epoxide (1.26%, only sample A), eudesmol (1.07%, only sample B) and other constituents traces (<1.0%). The major components of both oils were β-caryophyllene (51.62% and 57.16%), α-caryophyllene (8.42% and 8.94%), germacrene D (4.33% and 6.91%), biciclogermacrene (2.58% and 2.94%) and viridiflorene (2.46% and 2.09%). All samples were especially characterized by an abundance of sesquiterpenes hydrocarbons, especially with caryophyllane nucleus. These results are consistent with those reported for the oils obtained from the fruit of the species *C. grandiflora*, *C. minor*, *C. nemorosa* and *C. sandiensis* (Gonzáles et al., 1993; Oliveira et al., 2008). Terpenes cover a wide variety of substances from plants and its ecological importance in the defence of plants is well established (Viegas-Júnior, 2003). It is well known that β-caryophyllene, besides

Table 1
Chemical composition of essential oils of galled and non-galled leaves of *Clusia lanceolata*.

N°	KI[b]	Area[a] (%)		
		Compounds[c]	Galled leaves	Non-galled leaves
1	856	(2*E*)-hexenal	0.423	0.244
2	1338	δ-elemene	0.17	0.385
3	1381	α-copaene	1.074	0.881
4	1390	β-bourbonene	0.179	-
5	1395	β-elemene	0.689	0.433
6	1411	(*Z*)-caryophyllene	0.262	-
7	1423	β-caryophyllene	51.624	57.159
8	1436	β-copaene	0.286	0.134
9	1444	aromadendrene	0.622	0.498
10	1454	spirolepechinene	0.218	-
11	1463	α-caryophyllene	8.422	8.941
12	1467	*allo*-aromadendrene	5.430	6.389
13	1480	γ-muurolene	1.183	1.006
14	1487	germacrene D	4.333	6.906
15	1494	β-selinene	-	0.331
16	1497	viridiflorene	2.461	2.091
17	1502	bicyclogermacrene	2.582	2.942
18	1510	δ-amorphene	-	0.343
19	1517	γ-cadinene	0.671	0.563
20	1522	δ-cadinene	1.817	2.299
21	1562	germacrene B	0.815	0.974
22	1576	caryophyllenyl alcohol	-	0.316
23	1574	palustrol	0.532	-
24	1583	caryophyllene oxide	6.817	0.481
25	1591	globulol	0.642	0.432
26	1601	viridiflorol	0.833	-
27	1603	ledol	0.542	0.952
28	1611	eudesmol	-	1.065
29	1614	humulene 1,2-epoxide	1.263	-
30	1626	junenol	1.269	1.172
31	1647	*epi*-α-muurolol	0.899	0.905
32	1659	α-cadinol	1.532	1.293
Total			97.60	99.13

Notes: [a]Percentages based on FID peak area normalization.
[b]Retention index relative to *n*-alkanes (C_{8-20}) on the VF-5 MS capillary column.
[c]Compounds presented in order of elution from the VF-5 MS capillary column.

being anti-inflammatory, also has insecticidal and fungicidal activities (Arrhenius and Langenheim, 1983). Thus, this allowed us suggest that the presence of β-caryophyllene and other sesquiterpenes, as major chemical constituents in the essential oil of galled and non-galled leaves of this species, are alternatives to plant chemical defences against herbivory by galling promotion.

Total phenols, flavonoids and tannins in leaves of Clusia lanceolata

The low molecular weight polyphenols have a prominent role in plant protection against herbivores (insects and animals) (Harborne, 1999). The total phenol content of the methanol extracts from galled (311.2 ± 2.5 mg GAE. g^{-1}) and non-galled (199.4 ± 4.6 mg GAE. g^{-1}) leaves of *C. lanceolata* were significantly different ($p < 0.05$), higher in galled leaves. Motta et al. (2005) analyzed the total phenols in galled (15.99 ± 0.15) and non-galled (11.34 ± 0.03) leaves of *Tibouchina pulchra* and observed similar results, where the gall tissues showed higher total phenol content. Formiga et al. (2009) evaluated the relationship between total phenol content and cycle Cecidomyiidae galls on *Aspidosperma spruceanum* and noted that the phenols were deeply influenced by the induction and development of galls, which stimulated the production of these metabolites during the months analysed. The total flavonoid contents of galled (35.5 ± 0.5 mg QE.g^{-1}) and non-galled (54.8 ± 0.7 mg QE.g^{-1}) leaves of *C. lanceolata* were significantly different ($p < 0.05$), higher in healthy leaves. Similarly, the content of flavonoids in healthy leaves (1.26 ± 0.03) of *Tibouchina pulchra* was higher than the concentration present in the gall (0.31 ± 0.02) (Motta

et al., 2005). The presence of flavonoids was also observed by qualitative analysis of the extracts of galled and non-galled leaves in HPLC-DAD λ = 350 nm. The UV spectra (Fig. 1) of the main peaks exhibited two bands with maximum absorption around 270 and 350 nm, characteristic of flavone nucleus. The condensed tannins contents of galled (287 ± 1.9 mg CE.g^{-1}) and non-galled (61.1 ± 1.4 mg CE.g^{-1}) leaves were significantly different ($p < 0.05$), being higher in leaf galls. In tannin sensitive species, low levels of dietary tannins can have a variety of adverse consequences, ranging from reduced growth and abnormal development to death (Barbehein and Martin, 1994). Thus, it can be considered that the high content of tannins in the galled leaves of *C. lanceolata* is a response to herbivory by leaf promoted galling insects.

Characterisation of flavones C-glycosides

LC-ESI-MS/MS has been confirmed to be an ideal tool to analyse secondary metabolites from plants, owing to the fact that it can provide affluent multistage fragment information of compounds by collision-induced dissociation (Chen et al., 2009). Therefore, in our study, the high performance liquid chromatography with diode array detection and electrospray ionization ion trap mass spectrometry (HPLC-DAD-ESI-MS/MS) technique was employed for the identification of flavones in *C. lanceolata* leaves (Table 2). The four flavones (peaks 1, 2, 3 and 4 in Fig. 2) were identified as orientin, isoorientin, vitexin-2"-*O*-rhamnoside and isovitexin-2"-*O*-rhamnoside, respectively, by comparing the maximal UV wavelength, mass values of the $[M + H]^+$ and $[M - H]^-$ ion, as well as the fragmentation pathways in previous literature (Ying et al., 2007; Abad-García et al., 2008; Negri et al., 2012; Yao et al., 2012; Sun et al., 2013).

The ESI-MS spectra of peaks observed at 18.55 min (compound **1**) and 18.91 min (compound **2**) (Table 2, Fig. 2) showed the same (+) and (-)-ESI-MS spectra at *m/z* 449 and at *m/z* 447, respectively, suggesting that these compounds were isomers. Compound **1** and **2** were identified as orientin and isoorientin according to MS/MS data (Table 2) and fragmentation patterns (Fig. 3). Compound **2** showed the fragment ion at *m/z* 429 $[(M - H) - 18]^-$, 357 $[(M - H) - 90]^-$ and 327 $[(M - H) - 120]^-$, respectively. Compound **1** was associated the fragment ions at *m/z* 357 $[(M - H) - 90]^-$ and 327 $[(M - H) - 120]^-$. Among these fragment ions, *m/z* 429 $[(M - H) - 18]^-$ was only found in MS/MS of isoorientin, while it was not detected in orientin. Hence, the fragment ion $[(M - H) - 18]^-$ can be used to distinguish 6-*C*-glycosidic flavonoid from 8-*C*-glycosidic flavonoid (Abad-Garcia et al., 2008). In general, the fragmentation of the 6-*C*-isomers is more extensive, giving a ion corresponding to $[(M-H)-18]^-$, probably due to the formation of an additional hydrogen bond between the 2"-hydroxyl group of the sugar and the 5- or 7- hydroxyl group of the aglycone (Fig. 3), which confers additional rigidity (Cuyckens and Clalys, 2004; Negri et al., 2012).

Peaks 3 (t_R 20.17 min) and 4 (t_R 21.53 min) exhibited the similar maximum UV absorption (λ_{max} 270) and gave pseudomolecular ions at *m/z* 577 $[M - H]^-$. Peaks 3 and 4

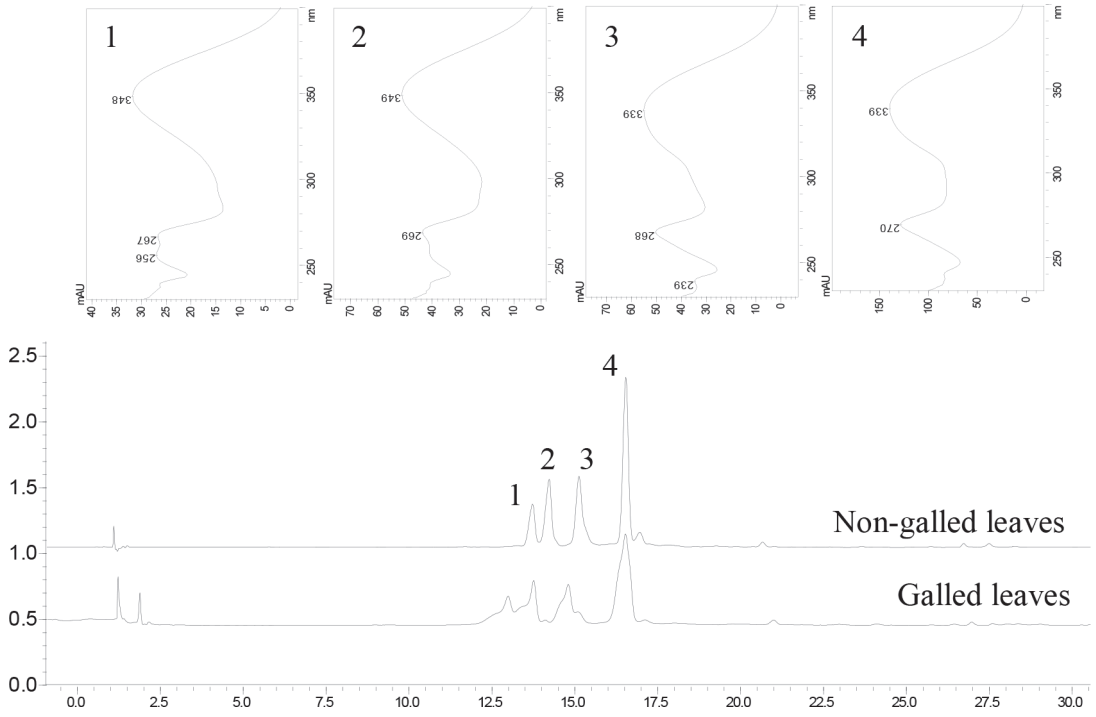

Figure 1 – Chromatograms (LC-DAD) of leaf extracts of *Clusia lanceolata* at 350 nm.

Table 2
Identification of phenolic compounds of leaves of *Clusia lanceolata* by LC-DAD and LC-ESI-MS/MS data.

Peak	t_R (min)	λmax (nm)	$[M+H]^+/[M-H]^-$	MS/MS (*m/z*) (ESI)$^-$	Compound identification
1	18.552	267, 348	449/447	357, 327	orientin
2	18.907	269, 349	449/447	429, 357, 327	isoorientin
3	20.170	268, 339	579/577	431, 413, 293	vitexin-2”-*O*-rhamnoside
4	21.531	270, 339	579/577	457, 431, 413, 293	isovitexin-2”-*O*-rhamnoside

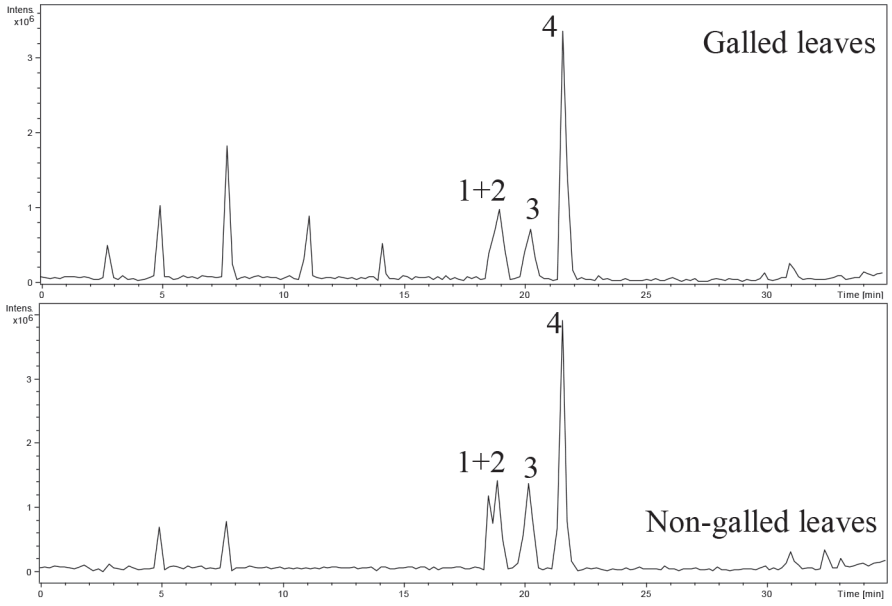

Figure 2 – The total current ion chromatograms (LC-ESI-MS) of the methanolic extract of *C. lanceolata* leaves in positive ion mode: (1) orientin, (2) isoorientin, (3) vitexin 2”-*O*-rhamnoside and (4) isovitexin 2”-*O*-rhamnoside.

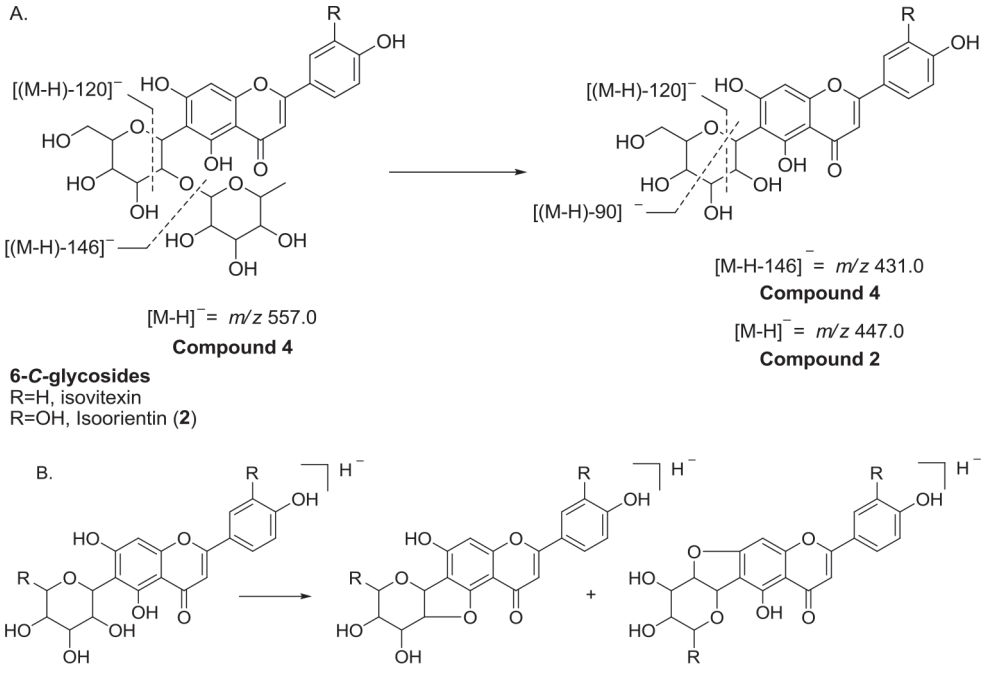

Figure 3 – A, Fragmentation of isovitexin-2”-*O*-rhamnoside and isoorientin. **B**, Loss of water observed for 6-*C*-glycosyl flavonoids involving the hydroxyl group at the 2”-position of the sugar residue and the hydroxyl group at the 5-or 7-position of the aglycone.

were identified as vitexin-2"-O-rhamnoside and isovitexin-2"-O-rhamnoside, according to MS/MS data (Table 2) and fragmentation patterns (Sun et al., 2013). Peak 4 was associated with fragment ions at *m/z* 457 $[(M - H) - 120]^-$, 431 $[(M - H) - 146]^-$, 413 $[(M - H) - 146 - 18]^-$ and 293 $[(M - H) - 284]^-$. Among these fragment ions, *m/z* 431 $[(M - H) - 146]^-$ can be explained by the loss of a rhamnose moiety and further fragmentation to an ion at *m/z* 413 $[(M - H) - 146 - 18]^-$, corresponding to the loss of a water molecule. Moreover, *m/z* 457 $[(M - H) - 120]^-$ resulted from a cross-ring cleavage of the *C*-glycosidic moiety and *m/z* 293 $[(M - H) - 284]^-$ was due to the combination of both fragmentations (Fig. 3). The compound associated with peak 4 was characterized as apigenin-6-*C*-glucosyl-2"-*O*-rhamnoside, also known as isovitexin-2"-*O*-rhamnoside.

The occurrence of *C*-glycosylated flavones has been described for other species of the genus *Clusia* (Delle Monache, 1991; Chedier et al., 1999; Compagnone et al., 2008). Thus, this study contributes to the knowledge of chemosystematics of this genus.

The LC-ESI-MS/MS (Fig. 2) analysis of *C. lanceolata* methanolic extracts provided two chemical profiles that allow us to distinguish between galled and non-galled leaves. Leaf galls of *C. lanceolata* have a greater number of detectable phenolic compounds than healthy leaves. These results suggest that this insect-plant interaction may be promoting changes in metabolism and thus in the distribution of metabolites in the leaves of *C. lanceolata* Cambess.

Antioxidant activity

The results of DPPH radical scavenging activity of the extract and the standard drug (ascorbic acid) are presented in Fig. 4. Extracts from galled leaves had higher antioxidant activity than non-galled leaves extracts ($p < 0.05$), but the antioxidant activity was significantly lower than the standard ascorbic acid ($p < 0.05$). Most reports indicated that the protective effect against oxidative damage of any samples or compounds was attributable to phenolic compounds (Robbins, 2003), and that part of the observed antioxidant activity can be attributed to *C*-glycosylated flavones identified in extracts from the leaves of *C. lanceolata*. The antioxidant activity of proanthocyanidins has been demonstrated to be fifty times greater than vitamin C and twenty times greater than vitamin E (Majo et al., 2008). The highest antiradical activity of galled leaves compared to healthy leaves may be related to higher phenolic content and proanthocyanidins. The variation in the distribution of phenolic metabolites resulting from this insect-plant interaction may be responsible for the higher antiradical activity of galled leaves.

The β-carotene/linoleic acid assay is a reproducible and authentic method to measure the antioxidant capacity of different biological samples. The enhanced capacity of the galled and non-galled leaves of *C. lanceolata* to bleach β-carotene in comparison with trolox (Fig. 4) confirms their greater antioxidant potential. The antioxidant activities of the galled and non-galled leaves in this study were similar and significantly higher than the standard trolox ($p < 0.05$), considering the concentrations used. The present study demonstrated that all evaluated extracts showed promising antioxidant activity and may act as a new source of natural antioxidants.

Authors' contributions

ROF and ARCJ contributed in collecting plant sample and identification, running the laboratory work, analysis of the data and drafted the paper. TMSS and TMGS contributed to chromatographic analysis. RNC contributed to antioxidant activity. MGC supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

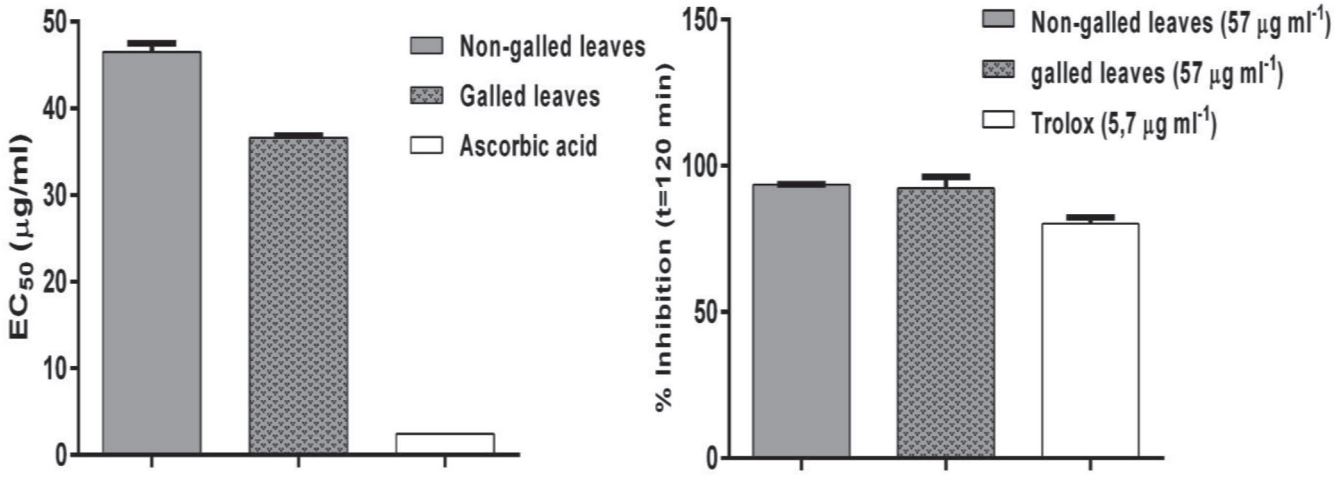

Figure 4 – The antioxidant activity of the extracts of *C. lanceolata* measured by DPPH and β-carotene/linoleic acid assays.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

The authors are grateful to Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro, CNPq, and CAPES for scholarships and financial support.

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