Phytochemistry 140 (2017) 45–51

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

# Isolation of antioxidant phenolics from *Schinopsis brasiliensis* based on a preliminary LC-MS profiling

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ARTICLE INFO

*Article history:*
Received 11 February 2017
Received in revised form
23 March 2017
Accepted 10 April 2017

*Keywords:*
*Schinopsis brasiliensis* (Anacardiaceae) stem bark
Phenolics
LC-MS profile
Cytotoxicity
Antioxidant activity

ABSTRACT

The phenolic content of the ethanol extract of the stem bark of the Brazilian plant *Schinopsis brasiliensis* Engl. (Anacardiaceae) has been evaluated together with the antioxidant activity. The good antioxidant activity exhibited in the Trolox Equivalent Antioxidant Capacity (TEAC) assay (TEAC value = 3.04 mg/mL) encouraged us to investigate its constituents. An analytical approach based on LC-ESIMS$^n$ was applied to rapidly obtain a metabolite profile of the ethanol extract of the stem bark of *S. brasiliensis*. Sixteen phenolic compounds, among which five galloyl derivatives, never reported before, have been isolated and their structures have been unambiguously elucidated by extensive spectroscopic methods, including 1D ($^1$H, $^{13}$C, TOCSY) and 2D (DQF-COSY, HMBC, and HSQC) NMR experiments. Moreover, the antioxidant activity of all the isolated compounds was evaluated, along with the cytotoxicity against the cancer cell lines A549 (human alveolar basal carcinoma) and Hela (human epitheloid cervix carcinoma). The previously undescribed compounds exhibited a high free-radical-scavenging activity, in the range of 1.10 −1.86 mM.

None of the tested compounds, in a range of concentrations between 12.5 and 100 μM, caused a significant reduction of the cell number.



## 1. Introduction

*Schinopsis brasiliensis* Engl. is a plant belonging to the Anacardiaceae family, known as “baraúna”, “bráuna” or “quebracho”, and characterized by arboreal habitat, with dimensions close to 15 m high. It can be found in the Brazilian semi-arid regions and it is a native species of the Caatinga and Atlantic forest (Fernandes et al., 2015). This plant is considered by the “First National Report for the Convention on Biological Diversity” (CBD) as an endangered species, due to the extensive utilisation of its wood in general construction (Cardoso et al., 2005).

Different parts of *S. brasiliensis*, including leaves, fruits, and mainly bark are used in Brazilian traditional medicine for the treatment of various diseases, such as inflammation, influenza, cough, diarrhea, dysentery, and as vermifuge (de Albuquerque et al., 2007; Junior et al., 2011). *S. brasiliensis* has also been used as a natural antiseptic to treat wounds and superficial mycoses (Saraiva et al., 2011).

Leaves and bark extracts from *S. brasiliensis* have been reported to possess antioxidant and antimicrobial activities (Saraiva et al., 2011), while a stem bark extract from *S. brasiliensis* and its fractions revealed molluscicidal and larvicidal activities (Santos et al., 2014).

Previous phytochemical investigations on *S. brasiliensis* leaves reported the presence of tannins and polyphenols, such as methyl gallate, gallic acid, ellagic acid derivatives (Fernandes et al., 2015), and flavonoids (Cardoso et al., 2015). Phytochemical investigation of the MeOH extract of *S. brasiliensis* stems showed the presence of a biflavonoid and a chalcone (Cardoso et al., 2015), while from *S. brasiliensis* bark an alkyl phenol along with a steroid derivative were isolated (Cardoso et al., 2005).

With the aim to validate some of the biological activities

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http://dx.doi.org/10.1016/j.phytochem.2017.04.008
0031-9422/

reported for *S. brasiliensis*, the phenolic content of the ethanol extract of the stem bark has been evaluated by Folin-Ciocalteau method together with the antioxidant activity by Trolox Equivalent Antioxidant Capacity (TEAC) assay (TEAC value = 3.04 mg/mL). The good antioxidant activity encouraged us to investigate its constituents. An analytical approach, based on LC-ESIMS$^n$, has been applied to rapidly obtain a metabolite profile of the ethanol extract of the stem bark of *S. brasiliensis*. It guided the isolation of 16 phenolic compounds, among which five previously unreported galloyl derivatives (**8**, **10**–**12** and **14**), which have been unambiguously elucidated by extensive spectroscopic methods, including 1D ($^1$H, $^{13}$C, TOCSY) and 2D NMR experiments (DQF-COSY, HMBC, and HSQC). The antioxidant activity of all isolated metabolites was also assayed.

Moreover, the cytotoxic activity of isolated compounds against the cancer cell lines A549 (human alveolar basal carcinoma) and Hela (human epitheloid cervix carcinoma) has been evaluated.

## 2. Results and discussion

The ethanol extract of *S. brasiliensis* stem bark collected in summer showed a high total phenol content determined by the Folin-Ciocalteau method and expressed as gallic acid equivalent (403.26 mg GAE/g of extract).

Aberrant production of reactive oxygen (ROS) or nitrogen (RNS) species has been demonstrated to contribute to the development of some prevalent diseases, including cancer and cardiovascular diseases. Polyphenols are able to react directly with ROS and RNS, preventing their concentrations from reaching harmful intracellular levels.

Thus the antioxidant activity of the ethanol extract of *S. brasiliensis* stem bark extract has been evaluated by TEAC assay, revealing a significant concentration-dependent free-radical scavenging activity (3.04 mg/mL).

Preliminary, LC-ESIMS$^n$ analysis in negative ionization mode of the ethanol extract of *S. brasiliensis* stem bark has been carried out (Fig. 1). Analysis of the LC-MS profile of the extract showed sixteen main peaks. The careful investigation of the multistage mass spectra of these compounds suggested their phenolic nature (Table 1). In particular, the careful study of the ESIMS$^n$ fragmentation pattern of each compound pointed out to assign common features to compounds **1**–**2**, **4**–**8**, **10**–**12** and **14**, showing the presence of a main ion fragment [M-H]$^-$ at *m/z* 169, corresponding to a galloyl moiety. Moreover, for compounds **2**, **5**, **6**, **8**, **10**, **12** and **14** additional main ion fragments [M-H]$^-$ at *m/z* 331 or at *m/z* 313 were evident, corresponding to the loss of a galloylhexose unit with or without a water molecule (Table 1).

**Table 1**
Retention times ($R_t$), Δ ppm, molecular formula, [M-H]$^-$ and MSMS values of compounds **1**–**16** of ethanol extract of *S. brasiliensis* stem bark by LC-ESI(Orbitrap)MS.

| | $R_t$ (min) | Δ ppm | Formula | [M-H]$^-$ | MSMS |
|---|---|---|---|---|---|
| **1** | 5.24 | 0.25 | $C_{13}H_{16}O_{10}$ | 331.0659 | 169 |
| **2** | 13.60 | −2.73 | $C_{20}H_{20}O_{14}$ | 483.0756 | 331, 169 |
| **3** | 14.71 | 2.80 | $C_{16}H_{24}O_9$ | 359.3668 | 197 |
| **4** | 14.73 | 1.40 | $C_{15}H_{20}O_{10}$ | 359.0968 | 169 |
| **5** | 14.88 | −3.09 | $C_{20}H_{22}O_{12}$ | 453.1013 | 313, 301, 169 |
| **6** | 14.88 | −2.47 | $C_{20}H_{22}O_{12}$ | 453.1016 | 313, 301, 169 |
| **7** | 15.07 | −2.59 | $C_{21}H_{20}O_{14}$ | 495.0756 | 343, 169 |
| **8** | 15.69 | −2.44 | $C_{21}H_{24}O_{12}$ | 467.1173 | 313,169 |
| **9** | 15.90 | 0.45 | $C_{15}H_{14}O_6$ | 289.0702 | 245,205,179 |
| **10** | 16.00 | −3.03 | $C_{20}H_{22}O_{12}$ | 453.2500 | 313, 301, 169 |
| **11** | 16.60 | −2.94 | $C_{24}H_{30}O_{13}$ | 525.1587 | 371, 169 |
| **12** | 17.46 | −2.34 | $C_{22}H_{24}O_{12}$ | 479.1173 | 331, 271, 169 |
| **13** | 18.43 | −1.54 | $C_{15}H_{14}O_6$ | 327.0020 | 165 |
| **14** | 19.33 | −2.44 | $C_{23}H_{28}O_{13}$ | 511.1434 | 359, 313, 169 |
| **15** | 19.35 | 3.03 | $C_{13}H_{18}O_8$ | 301.2833 | – |
| **16** | 19.64 | −2.90 | $C_{25}H_{32}O_{10}$ | 491.1896 | 169 |

In order to unambiguously elucidate their structures by NMR experiments, the ethanol extract was purified by size exclusion chromatography, followed by further purification steps by reversed-phase HPLC-RI, to obtain sixteen compounds. Their structures were established by 1D and 2D-NMR experiments along with ESIMS and HRESIMS analysis (Fig. 2).

A detailed analysis of NMR data of compounds **8**, **10**–**12**, and **14** showed the presence of a common moiety. For compound **8**, the $^1$H NMR spectrum showed an aromatic signal at $\delta$ 7.10 (2H, s),

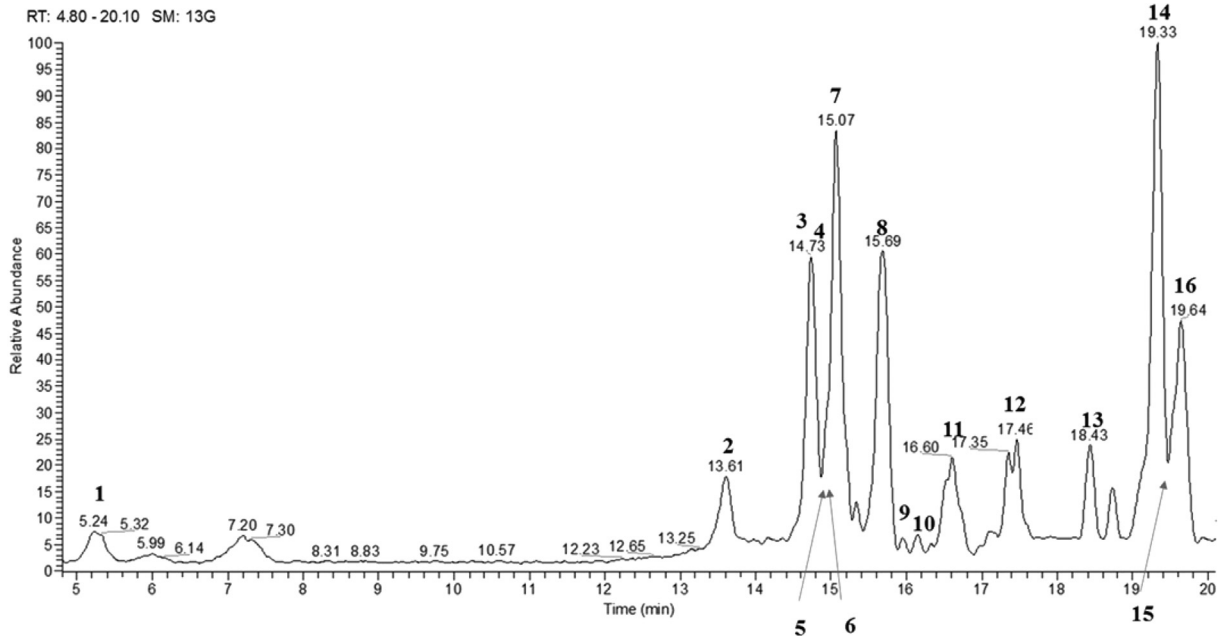


**Fig. 1.** LC-ESI(Orbitrap)MS profile (negative ion mode) of the ethanol extract of *S. brasiliensis* stem bark (collected in summer).

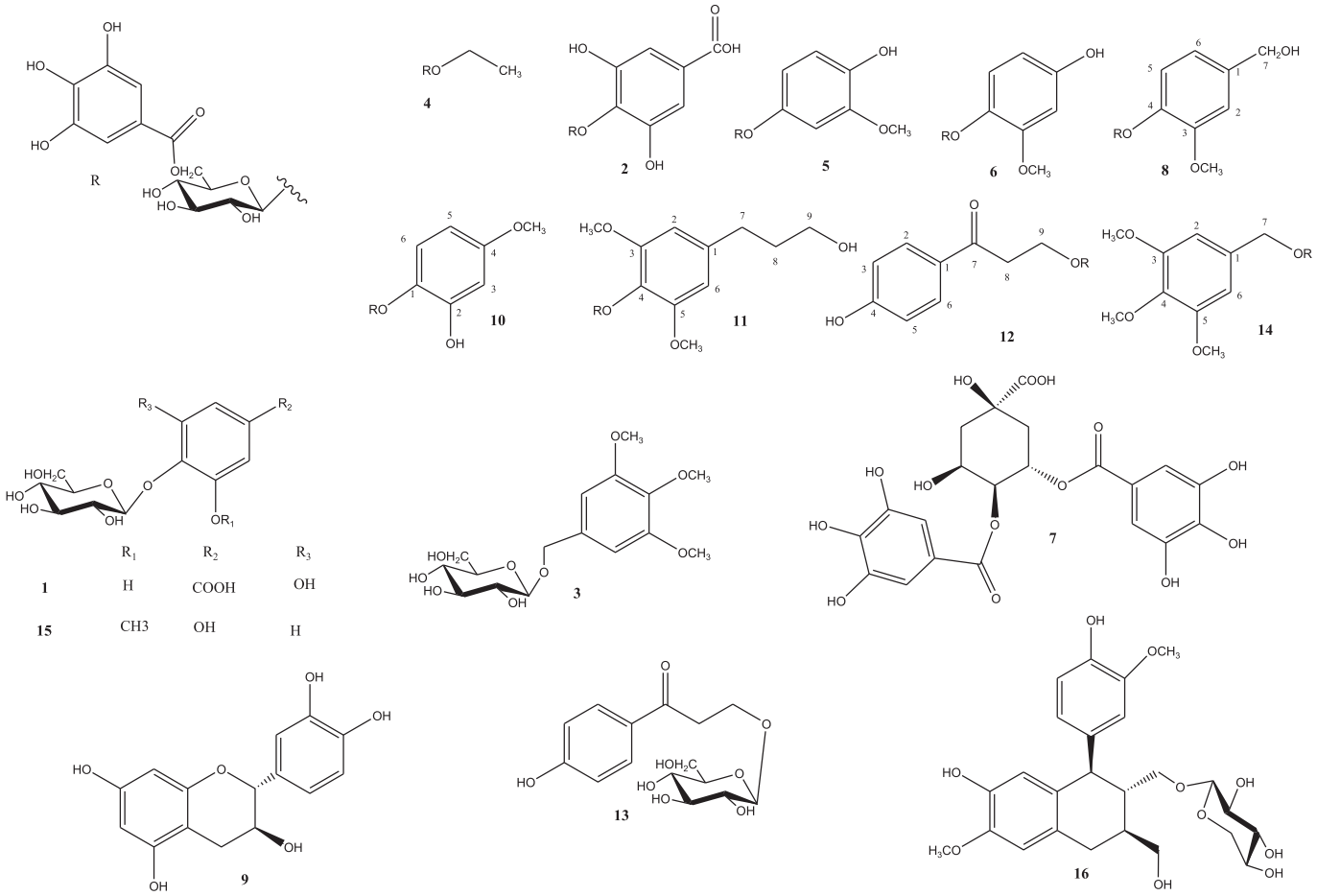

**Fig. 2.** Compounds isolated from *S. brasiliensis* stem bark.

ascribable to a galloyl moiety as confirmed by the HMBC correlation between the proton signal at $\delta$ 7.10 with the carbon resonances at $\delta$ 121.1 (C-1″), 146.4 (C-3″ and C-5″), 139.5 (C-4″) and 167.9 (C-7″) (Table 2). Moreover, for the sugar unit an anomeric signal at $\delta$ 4.89 (d, $J$ = 7.8 Hz) was evident. On the basis of HSQC, HMBC, COSY and 1D-TOCSY experiments a β-glucopyranosyl unit was identified. The absolute configuration of the sugar unit was determined by acid hydrolysis of compound **8** and was assigned as D-glucose on the basis of its optical rotation value (Horo et al., 2015). The downfield shifts of protons at $\delta$ 4.60 and 4.43 ($H_2$-6′) of the primary alcoholic function of glucose and their HMBC correlation with the carbon resonance at $\delta$ 167.9 (C-7″) revealed the site of esterification. The β-D-glucopyranosyl-(6-*O*-galloyl)-unit was also identified in the NMR spectra of compounds **10**–**12** and **14**.

The HRESIMS of compound **8** (*m/z* 467.1190 [M-H]$^-$, calcd for $C_{21}H_{23}O_{12}$, 467.1173) and the $^{13}$C NMR data supported a molecular formula of $C_{21}H_{24}O_{12}$. The $^1$H NMR spectrum showed additional aromatic signals at $\delta$ 7.07 (d, $J$ = 8.0 Hz), 7.03 (d, $J$ = 1.8 Hz) and 6.76 (dd, $J$ = 1.8, 8.0 Hz) typical of a 1,3,4-trisubstituted aromatic ring, at $\delta$ 4.53 (2H, s) ascribable to a primary alcoholic group and at $\delta$ 3.88 (3H, s), corresponding to a methoxy group (Table 2). The HMBC correlations between the proton signal at $\delta$ 7.03 (H-2) with the carbon signals at $\delta$ 137.4 (C-1), 150.4 (C-3), 146.6 (C-4) and 64.9 (C-7) and the cross-peak between the methoxy function at $\delta$ 3.88 with the carbon resonance at δ 150.4 (C-3) allowed us to identify the aglycone as vanillyl alcohol. A further HMBC correlation between the anomeric signal at $\delta$ 4.89 with the carbon resonance at $\delta$ 146.6 (C-4) established the structure of **8** as the previously unreported vanillyl alcohol-4-*O*-β-D-(6′-*O*-galloyl)-glucopyranoside.

The HRESIMS of **10** (*m/z* 453.1033 [M-H]$^-$, calcd for $C_{20}H_{21}O_{12}$, 453.2500) and the $^{13}$C NMR data supported a molecular formula of $C_{20}H_{22}O_{12}$. The $^1$H NMR spectrum showed along with the signals ascribable to a β-D-glucopyranosyl-(6-*O*-galloyl)-unit, signals at $\delta$ 6.98 (d, $J$ = 8.3 Hz), 6.46 (d, $J$ = 1.8 Hz) and 6.20 (dd, $J$ = 1.8, 8.3 Hz) typical of a 1,3,4-trisubstituted aromatic ring, and a signal at $\delta$ 3.82 (3H, s), corresponding to a methoxy group, assigned by the HMBC correlation to C-4 (Table 2). Thus, the aglycone of **10** was identified as 2-hydroxy-4-methoxyphenol. A further HMBC correlation between the anomeric signal at $\delta$ 4.70 with the carbon resonance at $\delta$ 141.3 (C-1) allowed to establish the structure of **10** as the previously unreported 2-hydroxy-4-methoxyphenol-1-*O*-β-D-(6′-*O*-galloyl)-glucopyranoside. A similar compound, the 2-hydroxy-4-methoxyphenol 1-*O*-β-D-(6′-*O*-syringoyl)-glucopyranoside, differing for the presence of methoxy group on the galloyl unit, was reported in *Juglans mandshurica* (Machida et al., 2009).

The molecular formula of **11** was established as $C_{24}H_{30}O_{13}$ by HRESIMS (*m/z* 525.1608 [M-H]$^-$, calcd for $C_{24}H_{29}O_{13}$, 525.1587) and $^{13}$C NMR data. The $^1$H NMR spectrum showed along with the signals ascribable to a β-D-glucopyranosyl-(6-*O*-galloyl)-unit, an aromatic signal at $\delta$ 6.48 (2H, s) and a signal at $\delta$ 3.78 (6H, s) typical of two methoxy groups, assigned on the basis of 2D-NMR data to a 1,3,4,5-tetrasubstituted aromatic ring. Further signals in the proton spectrum at $\delta$ 3.52 (2H, t, $J$ = 7.2 Hz), 2.61 (t, $J$ = 8.0 Hz) and 1.81 (2H, q, $J$ = 8.0 Hz), suggested the presence of a propanol unit, as confirmed by the linear connectivity observed in the COSY spectrum (Table 2). The HMBC correlation between the proton signals at $\delta$ 2.61 and 1.81

**Table 2**
$^{1}$H NMR spectroscopic data (600 MHz, *J* in Hz) and $^{13}$C NMR (150 MHz) data of compounds **8**, **10**–**12** and **14** ($CD_3OD$).

| | **8** | | **10** | | **11** | | **12** | | **14** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1 | 137.4 | – | 141.3 | – | 140.0 | – | 129.8 | – | 134.5 | – |
| 2 | 114.8 | 7.03, d (1.8) | 154.7 | – | 107.0 | 6.48, s | 131.7 | 7.94, d (8.0) | 105.9 | 6.70, s |
| 3 | 150.4 | – | 101.3 | 6.46, d (1.8) | 153.8 | – | 115.9 | 6.87, d (8.0) | 154.3 | – |
| 4 | 146.6 | – | 151.8 | – | 133.7 | – | 163.0 | – | 138.2 | – |
| 5 | 117.7 | 7.07, d (8.0) | 107.3 | 6.20, dd (1.8, 8.3) | 153.8 | – | 115.9 | 6.87, d (8.0) | 154.3 | – |
| 6 | 120.7 | 6.76, dd (1.8, 8.0) | 120.3 | 6.98, d (8.3) | 107.0 | 6.48, s | 131.7 | 7.94, d (8.0) | 105.9 | 6.70, s |
| 7 | 64.9 | 4.53, s | – | – | 33.0 | 2.61, t (8.0) | 199.1 | – | 71.2 | 4.80, d (12.0)<br>4.64 d (12.0) |
| 8 | – | – | – | – | 35.0 | 1.81, q (8.0) | 39.0 | 3.33, overlapped | | |
| 9 | – | – | – | – | 61.8 | 3.52, t (7.2) | 66.4 | 4.27 dd (12.0, 7.0),<br>4.07 dd (12.0, 7.0) | | |
| $OCH_3$ | 56.4 | 3.88, s | 56.0 | 3.82, s | 56.4 | 3.78, s | | | 56.2 | 3.80, s |
| $OCH_3$ | | | | | 56.4 | 3.78, s | | | 55.7 | 3.75, s |
| $OCH_3$ | | | | | | | | | 56.2 | 3.80, s |
| | β-glucopyranosyl | | β-glucopyranosyl | | β-glucopyranosyl | | β-glucopyranosyl | | β-glucopyranosyl | |
| 1′ | 102.6 | 4.89, d (7.8) | 103.8 | 4.70, d (7.8) | 105.0 | 4.80, d (7.8) | 104.7 | 4.39, d (7.8) | 102.4 | 4.36, d (7.8) |
| 2′ | 74.6 | 3.56, dd (7.8, 9.0) | 74.3 | 3.50, dd (7.8, 9.0) | 75.0 | 3.57, dd (7.8, 9.0) | 74.9 | 3.22, dd (7.8, 9.0) | 74.0 | 3.32, dd (7.8, 9.0) |
| 3′ | 77.4 | 3.52, dd (9.0, 9.0) | 77.2 | 3.50, dd (9.0, 9.0) | 77.6 | 3.49, dd (9.0, 9.0) | 77.6 | 3.45, dd (9.0, 9.0) | 77.6 | 3.41, dd (9.0, 9.0) |
| 4′ | 71.5 | 3.47 dd (9.0, 9.0) | 71.1 | 3.50 dd (9.0, 9.0) | 71.4 | 3.45 dd (9.0, 9.0) | 71.6 | 3.43 dd (9.0, 9.0) | 71.7 | 3.45 dd (9.0, 9.0) |
| 5′ | 75.5 | 3.73 m | 75.1 | 3.67, m | 75.3 | 3.50, m | 75.3 | 3.58, m | 74.8 | 3.56, m |
| 6′ | 64.5 | 4.60, dd (2.5, 12.0)<br>4.43, dd (4.5, 12.0) | 64.5 | 4.58, dd (2.5, 12.0)<br>4.47, dd (4.5, 12.0) | 64.0 | 4.57, dd (2.5, 12.0)<br>4.46, dd (4.5, 12.0) | 64.5 | 4.51, dd (2.5, 12.0)<br>4.41, dd (4.5, 12.0) | 64.5 | 4.61, dd (2.5, 12.0)<br>4.45, dd (4.5, 12.0) |
| | galloyl unit | | galloyl unit | | galloyl unit | | galloyl unit | | galloyl unit | |
| 1″ | 121.1 | – | 121.1 | – | 121.1 | – | 121.1 | – | 120.9 | – |
| 2″ | 109.8 | 7.10, s | 110.2 | 7.13, s | 109.8 | 7.03, s | 109.7 | 7.12, s | 109.8 | 7.14, s |
| 3″ | 146.4 | – | 146.4 | – | 146.2 | – | 146.4 | – | 146.4 | – |
| 4″ | 139.5 | – | 139.9 | – | 139.7 | – | 139.7 | – | 139.6 | – |
| 5″ | 146.4 | – | 146.4 | – | 146.2 | – | 146.4 | – | 146.4 | – |
| 6″ | 109.8 | 7.10, s | 110.2 | 7.13, s | 109.8 | 7.03, s | 109.7 | 7.12, s | 109.8 | 7.14, s |
| 7″ | 167.9 | – | 167.9 | – | 167.9 | – | 168.2 | – | 167.9 | – |

with the carbon resonances at *δ* 140.0 (C-1) and 107.0 (C-2, C-6) prompted us to identify the aglycone of **11** as the dihydrosinapyl alcohol. Finally, the HMBC correlation between the anomeric proton at *δ* 4.80 with the carbon resonance at *δ* 133.7 (C-4) established compound **11** as the previously unreported dihydrosinapyl alcohol-4-*O*-(6′-*O*-galloyl)-β-D-glucopyranoside.

The HRESIMS mass spectrum of **12** (*m/z* 479.1190 [M-H]$^-$, calcd for $C_{22}H_{23}O_{12}$, 479.1173) supported a molecular formula of $C_{22}H_{24}O_{12}$. In addition to the signals corresponding to the β-D-glucopyranosyl-(6-*O*-galloyl) moiety, further signals in the aromatic region at *δ* 7.94 (2H, d, *J* = 8.0) and 6.87 (2H, d, *J* = 8.0) typical of a 1,4-disubstituted aromatic ring, at *δ* 4.27 (dd, *J* = 12.0, 7.0) and 4.07 (dd, *J* = 12.0, 7.0) ascribable to a primary alcoholic function and at *δ* 3.18 (overlapped) were evident in the $^{1}$H NMR spectrum of compound **12** (Table 2). Analysis of NMR data allowed the structure of the aglycone of **12** to be established as 4,9-dihydroxypropiophenone. The HMBC correlation between the anomeric signal at *δ* 4.39 (H-1′$_{glc}$) with the carbon resonance at *δ* 66.4 (C-9) allowed to assign unambiguously the site of linkage of the sugar moiety. Consequently, the structure of **12** was determined as the previously unreported 4,9-dihydroxypropiophenone-9-*O*-(6′-*O*-galloyl)-β-D-glucopyranoside.

The HRESIMS mass spectrum of **14** (*m/z* 511.1452 [M-H]$^-$, calcd for $C_{23}H_{27}O_{13}$, 511.1434) showed a molecular formula of $C_{23}H_{28}O_{13}$, also supported by the $^{13}$C NMR data. The $^{1}$H NMR spectrum showed along with the signals ascribable to a β-D-glucopyranosyl-(6-*O*-galloyl)-unit, signals at *δ* 6.70 (2H, s), 3.80 (6H, s), and 3.75 (3H, s) assigned on the basis of 2D-NMR data to a 3,4,5-trimethoxyphenyl ring, and at *δ* 4.80 and 4.64 (each, d, *J* = 12.0 Hz), ascribable to a primary alcoholic group (Table 2). The HMBC correlation between the proton signals at *δ* 4.80 and 4.64 with the carbon resonances at *δ* 134.5 (C-1) and 105.9 (C-2, C-6) prompted us to identify the aglycone of **14** as 3,4,5-trimethoxybenzyl alcohol. Finally, the HMBC correlation between the anomeric proton at *δ* 4.36 with the carbon resonance at *δ* 71.2 (C-7) established compound **14** as the previously unreported 3,4,5- trimethoxybenzyl alcohol-7-*O*-(6-*O*-galloyl)-β-D-glucopyranoside.

Comparison with NMR data reported in literature allowed us to identify the known compounds as gallic acid 4-*O*-β-D-glucopyranoside (**1**) (Fotiric Aksic et al., 2015), gallic acid 4-*O*-*β*-D-(6′-*O*-galloyl)-glucopyranoside (**2**) (Lee et al., 2011), nikoenoside (**3**) (Meng et al., 2010), ethyl-*O*-*β*-D-(6′-*O*-galloyl)-glucopyranoside (**4**) (Kang et al., 2008), 4-hydroxy-3-methoxyphenol-1-*O*-(6′-*O*-galloyl)-β-D-glucopyranoside (**5**) (Shi et al., 2010), 4-hydroxy-2-methoxyphenol-1-*O*-*β*-D-(6′-*O*-galloyl) glucopyranoside (**6**) (Saijo et al., 1989), 3,4-di-*O*-galloyl-quinic acid (**7**) (Zhu et al., 2012), catechin (**9**) (Benavides et al., 2006), 4,9-dihydroxypropiophenone-9-*O*-β-D-glucopyranoside (**13**) (Meng et al., 2010), 4-hydroxy-2-methoxyphenol-1-*O*-*β*-D-glucopyranoside (**15**) (Shi et al., 2010) and schizandriside (**16**) (Kim et al., 2012).

Thus, in addition to previously unreported compounds (**8**, **10**–**12**, **14**), also compounds **2**, **4**–**6** possess a β-D-glucopyranosyl-(6-*O*-galloyl)-moiety. The above cited compounds along with compounds **1**, **3** and **15**, possessing a phenolic ring, and catechin (**9**) and lignan (**16**) justify the high phenolic content and the high antioxidant activity exhibited by the extract of *S. brasiliensis*.

With the aim to investigate if the qualitative profile of the extract of *S. brasiliensis* was affected by season changes, the LC-ESIMS$^n$ analysis, in negative ionization mode, of the ethanol extract of the stem bark collected in winter has been performed. No changes in the qualitative profile have been observed in the winter extract, evidencing that seasonality is not a factor that influences the occurrence of chemical constituents of the plant (See Supporting Information).

The antioxidant activity of compounds **1**–**16** was tested by TEAC assay and expressed as TEAC value, defined as the

concentration of Trolox solution with antioxidant capacity equivalent to a 1 mM concentration of the test sample (Pereira do Amaral et al., 2012) (Table 3), and compared to that of quercetin 3-*O*-glucopyranoside. The results showed that most of isolated compounds (**2**, **5**–**7**, **10** and **12**) exhibited higher free-radical-scavenging activity than quercetin 3-*O*-glucopyranoside. Compounds **8**, **10**–**12** and **14** never reported before exhibited a good free-radical-scavenging activity, in the range of 1.10–1.86 mM.

The ability of several antioxidant plant phenolics to reduce the risk and slow the progression of some cancer diseases by prevention of cell oxidation has been widely reported (Pereira do Amaral et al., 2012). Thus, the cytotoxic activity of compounds **1**–**16** was tested against two cancer cell lines, A549 and Hela, by the MTT assay. None of the tested compounds, in a range of concentrations between 12.5 and 100 μM, caused a significant reduction of the cell number (data not shown).

## 3. Experimental section

### 3.1. General instrumentation

IR measurements were obtained on a Bruker IFS-48 spectrometer and UV measurements were recorded on a UV–visible spectrophotometer (Evolution 201, Thermo Fisher Scientific). NMR experiments were performed on a Bruker DRX-600 spectrometer (Bruker BioSpin GmBH, Rheinstetten, Germany) equipped with a Bruker 5 mm TCI CryoProbe at 300 K. All 2D-NMR spectra were acquired in methanol-$d_4$ (99.95%, Sigma-Aldrich) and standard pulse sequences and phase cycling were used for DQF-COSY, HSQC, and HMBC spectra. The NMR data were processed using TOPSPIN 3.2 software. HPLC separations were carried out on a Waters 590 system equipped with a Waters R401 refractive index detector, a Waters XTerra Prep MSC18 column (300 × 7.8 mm i.d.), and a Rheodyne injector. HRESIMS spectra were carried out by an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) operating in negative ion mode. The Orbitrap mass analyzer was calibrated according to the manufacturer's directions using a mixture of caffeine, methionine-arginine-phenylalanine-alanine-acetate (MRFA), sodium dodecyl sulfate, sodium taurocholate and Ultramark 1621. Data were collected and analyzed using the software provided by the manufacturer.

**Table 3**
Free radical scavenging activities of compounds **1**–**16** and the ethanol extract of *S. brasiliensis* stem bark in the TEAC assay.

| Compounds | TEAC value (mM± SD)[a] |
|---|---|
| **1** | 1.24 ± 0.07 |
| **2** | 1.97 ± 0.05 |
| **3** | 0.63 ± 0.06 |
| **4** | 1.21 ± 0.05 |
| **5** | 1.83 ± 0.01 |
| **6** | 1.67 ± 0.01 |
| **7** | 1.83 ± 0.01 |
| **8** | 1.48 ± 0.01 |
| **9** | 1.44 ± 0.09 |
| **10** | 1.86 ± 0.08 |
| **11** | 1.41 ± 0.01 |
| **12** | 1.58 ± 0.01 |
| **13** | 0.79 ± 0.01 |
| **14** | 1.10 ± 0.04 |
| **15** | 1.08 ± 0.01 |
| **16** | 1.21 ± 0.01 |
| Quercetin 3-*O*-glucopyranoside | 1.54 ± 0.01 |
| | TEAC value (mg/mL ± SD) |
| Ethanol extract | 3.04 ± 0.02 |

[a] The TEAC value is the concentration of a standard Trolox solution with the same antioxidant capacity as a 1 mg/mL of the tested sample.

### 3.2. Plant material

The stem bark of *Schinopsis brasiliensis* Engl. (Anacardiaceae) was collected between February and August 2014 in summer and winter period, respectively in the city of Piranhas in Alagoas, Brazil, whose geographical coordinates were identified by a Garmin Forerunner GPS function (9°35′54.37″ S and 37°46′08.31″ W). The species was identified by the biologist Marta Maria Cristina Farias and a voucher specimen (24442 ASE) was deposited at the herbarium of the Department of Biology of the Federal University of Sergipe.

### 3.3. Extraction procedure

The stem bark of *S. brasiliensis* (4 Kg) was dried at room temperature, reduced to powder and extracted at room temperature using ethanol-water (9:1) for five days. After filtration and evaporation of the solvent to dryness in vacuo, 420 g of hydralcoholic extract were obtained.

### 3.4. Determination of total phenol content (Folin–Ciocalteu method)

The ethanol extract of *S. brasiliensis* stem bark, collected in summer, was analyzed according to the Folin-Ciocalteu (FC) colorimetric method (Shahidi et al., 2007). The ethanol extract was dissolved in MeOH to obtain a concentration of 0.5 mg/mL. Folin-Ciocalteu phenol reagent (0.5 mL) was added to centrifuge tubes containing 0.5 mL of the extracts. The contents were mixed, and 1 mL of a saturated sodium carbonate solution was added to each tube, to arrive to the volume of 10 mL with distilled water. The contents in the tubes were mixed by vortex, kept at room temperature for 45 min (until the characteristic blue color developed) and then centrifuged at 3000 rpm for 5 min. Absorbance of the clear supernatant was measured at 517 nm on a UV–visible spectrophotometer (Evolution 201, Thermo Fisher Scientific). A control without FC reagent and a blank with methanol instead of sample were included in the assay. The total polyphenol content was expressed as gallic acid equivalents (mg GAE/g extract, means ± SD of three determinations) calculated by calibration curves ($y = 0.0027x+0.0982$ $R^2 = 0.9929$).

### 3.5. Antioxidant activity (TEAC assay)

The antioxidant activity of the ethanol extract of *S. brasiliensis* stem bark, collected in summer, was determined by the Trolox Equivalent Antioxidant Capacity (TEAC) assay as previously reported (Kirmizibekmez et al., 2012). The TEAC value is based on the ability of the antioxidant to scavenge the radical cation 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) $ABTS^{\bullet+}$ by spectrophotometric analysis. The $ABTS^{\bullet+}$ cation radical was produced by the reaction between 7 mM ABTS in $H_2O$ and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 h. $ABTS^{\bullet+}$ is a blue-green chromogen with a characteristic absorption at 734 nm. The $ABTS^{\bullet+}$ solution was then diluted with PBS (phosphate saline buffer, pH = 7.4) to obtain an absorbance of 0.70 at 734 nm and equilibrated at 30 °C.

The extract was diluted with methanol to produce solutions of 250, 500, 750, 1000 μg/mL. Samples **1**–**16** were diluted with methanol to produce solutions of 0.3, 0.5, 1 and 1.5 mM. The reaction was initiated by the addition of 1.5 mL of diluted ABTS to 15 μL of each sample solution. Determinations were repeated three times for each sample solution. The inhibition percentage of

absorbance at 734 nm was calculated for each concentration relative to a blank absorbance (methanol) and was plotted as a function of concentration of compound or standard, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, Aldrich Chemical Co., Gillingham, Dorset, UK). The antioxidant activities are expressed as TEAC values in comparison with TEAC activity of quercetin 3-*O*-glucopyranoside, used as reference compound. The TEAC value is defined as the concentration of a standard Trolox solution with the same antioxidant capacity as a 1 mg/mL of the tested extract.

### 3.6. LC-ESI(Orbitrap)MS analysis

Qualitative LC-MS was performed using a Thermo Scientific Accela HPLC system (Thermo Scientific, Germany) equipped with a $C_{18}$ reversed-phase (RP) column (2.1 × 250 mm; X-Terra MS C18 5 lm; Waters, Milford, MA) at a flow rate of 0.2 μl/min and coupled to a LTQ-Orbitrap XL mass spectrometer. The gradient elution was carried out by using 0.1% formic acid as eluent A and acetonitrile as B. The HPLC gradient started at 5% (B) hold for 5 min and after 50 min, %B was at 100% holding it for 5 min before returning back to the starting percentage. The instrument was calibrated using the manufacturer's calibration standards. The scan was collected in the Orbitrap at a resolution of 30 000 in a *m/z* range of 230–1500 amu. The *m/z* of each identified compound was calculated to 4 decimal places and measured with a mass accuracy <3.5 ppm. The source voltage was 4.0 kV and capillary voltage −35 kV, the tube lens offset −126 V and the capillary temperature was set at 280 °C, the auxiliary gas was set at 10 (arbitrary units) and the sheath gas at 20 (arbitrary units). In full LC-ESIMS experiments Total Ion Current (TIC) profile was produced by monitoring the intensity of all the ions produced and acquired in every scan during the chromatographic run. In order to get structural information, Data Dependent experiments were performed by acquiring $MS^2$ spectra of the most intense ions produced during the acquisition.

### 3.7. Isolation procedure

The hydroalcoholic extract was dried under vacuum and 3 g were fractionated on a Sephadex LH-20 (Pharmacia) column (100 × 5 cm), using MeOH as mobile phase, affording 91 fractions (8 mL), monitored by TLC.

Fraction 14–18 (111.0 mg) were chromatographed by semipreparative HPLC using MeOH-$H_2O$ (3:7) as mobile phase (flow rate 2.5 mL/min) to yield compounds **3** (6.3 mg, $t_R$ = 4.8 min), **11** (5.8 mg, $t_R$ = 10.0 min) and **16** (8.2 mg, $t_R$ = 48.0 min). Fractions 19–25 (134.3 mg) were chromatographed by semipreparative HPLC using MeOH-$H_2O$ (3:7) as mobile phase (flow rate 2.5 mL/min) to yield compounds **8** (8.6 mg, $t_R$ = 10.5 min) and **14** (10.2 mg, $t_R$ = 46.0 min). Fractions 26–31 (130.9 mg) were chromatographed by semipreparative HPLC using MeOH-$H_2O$ (1:3) as mobile phase (flow rate 2.5 mL/min) to yield compounds **4** (6.6 mg, $t_R$ = 10.0 min), **5** (5.1 mg, $t_R$ = 16.2 min) and **6** (5.7 mg, $t_R$ = 15.8 min). Fractions 31–32 (103.5 mg) were chromatographed by semipreparative HPLC using MeOH-$H_2O$ (3:7) as mobile phase (flow rate 2.5 mL/min) to yield compound **10** (4.8 mg, $t_R$ = 11.5 min). Fractions 37–39 (31.8 mg) were chromatographed by semipreparative HPLC using MeOH-$H_2O$ (3:7) as mobile phase (flow rate 2.5 mL/min) to yield compound **9** (5.0 mg, $t_R$ = 10.4 min). Fraction 48 (32.4 mg) were chromatographed by semipreparative HPLC using MeOH-$H_2O$ (3:7) as mobile phase (flow rate 2.5 mL/min) to yield compounds **2** (5.5 mg, $t_R$ = 9.0 min) and **7** (9.4 mg, $t_R$ = 43.0 min).

Fractions 64–74 (105.9 mg) were chromatographed by semipreparative HPLC using MeOH-$H_2O$ (3:7) as mobile phase (flow rate 2.5 mL/min) to yield compounds **12** (5.2 mg, $t_R$ = 26.0 min) **13** (5.9 mg, $t_R$ = 30.0 min) and **15** (5.7 mg, $t_R$ = 36.2 min), Fractions 90–91 (65.9 mg) were chromatographed by semipreparative HPLC using MeOH-$H_2O$ (3:7) as mobile phase (flow rate 2.5 mL/min) to yield compound **1** (10.1 mg, $t_R$ = 10.0 min).

### 3.8. Vanillyl alcohol 4-O-β-D-(6′-O-galloyl) glucopyranoside (*8*)

Amorphous white solid; $C_{21}H_{24}O_{12}$; IR $\nu^{KBr}_{max}$ $cm^{-1}$: 3450, 2930, 1785, 1660; $^1H$ and $^{13}C$ NMR (methanol-$d_4$, 600 MHz) data, see Table 2; HRESIMS *m/z* 467.1190 $[M-H]^-$ (calcd for $C_{21}H_{23}O_{12}$, 467.1173).

### 3.9. 2-Hydroxy-4-methoxyphenol 1-O-β-D-(6′-O-galloyl) glucopyranoside (*10*)

Amorphous white solid; $C_{20}H_{22}O_{12}$; IR $\nu^{KBr}_{max}$ $cm^{-1}$: 3430, 2945, 1765, 1675; $^1H$ and $^{13}C$ NMR (methanol-$d_4$, 600 MHz) data, see Table 2; HRESIMS *m/z* 453.1033 $[M-H]^-$ (calcd for $C_{20}H_{21}O_{12}$, 453.2500).

### 3.10. Dihydrosinapyl alcohol 4-O-(6′-O-galloyl)-β-D-glucopyranoside (*11*)

Amorphous white solid; $C_{24}H_{30}O_{13}$; IR $\nu^{KBr}_{max}$ $cm^{-1}$: 3420, 2950, 1750, 1685, 1130; $^1H$ and $^{13}C$ NMR (methanol-$d_4$, 600 MHz) data, see Table 2; HRESIMS *m/z* 525.1608 $[M-H]^-$ (calcd for $C_{24}H_{29}O_{13}$, 525.1587).

### 3.11. 4,9-Dihydroxypropiophenone-9-O-(6′-O-galloyl)-β-D-glucopyranoside (*12*)

Amorphous white solid; $C_{22}H_{24}O_{12}$; IR $\nu^{KBr}_{max}$ $cm^{-1}$: 3410, 2930, 1770, 1715, 1680, 1200; $^1H$ and $^{13}C$ NMR (methanol-$d_4$, 600 MHz) data, see Table 2; HRESIMS *m/z* 479.1190 $[M-H]^-$ (calcd for $C_{22}H_{23}O_{12}$, 479.1173).

### 3.12. 3,4,5- trimethoxybenzyl alcohol 7-O-(6′-O-galloyl)-β-D-glucopyranoside (*14*)

Amorphous white solid; $C_{23}H_{28}O_{13}$; IR $\nu^{KBr}_{max}$ $cm^{-1}$: 3400, 2925, 1775, 1720, 1685, 1135; $^1H$ and $^{13}C$ NMR (methanol-$d_4$, 600 MHz) data, see Table 2; HRESIMS *m/z* 511.1452 $[M-H]^-$ (calcd for $C_{23}H_{27}O_{13}$, 511.1434).

### 3.13. Acid hydrolysis

A mixture of compounds **8** (5 mg), **10** (3 mg), **11** (3 mg), **12** (3 mg) and **14** (5 mg) was heated at 60 °C with 1:1 0.5 N HCl-dioxane (3 mL) for 2 h, and then evaporated in vacuo. The solution was partitioned with $CH_2Cl_2$-$H_2O$, and the $H_2O$ layer was neutralized with Amberlite MB-3. The $H_2O$ layer was then concentrated and passed through a silica gel column, using $CHCl_3$-MeOH-$H_2O$ (7:1:1.2, lower layer) as eluting solvent to afford glucose. The D configuration of glucose was established by comparison of its optical rotation value with those reported in the literature (Horo et al., 2015). The optical rotation was determined after dissolving the sugars in $H_2O$ and allowing them to stand for 24 h; D-glucose $[\alpha]^{22}_D$ +47.9 (*c* 0.1).

### 3.14. Cancer cell lines

Human alveolar basal carcinoma (A549), obtained from the European Collection of Cell Cultures (ECACC), and HeLa cells

(human epitheloid cervix carcinoma), obtained from Cell Bank in GMP-IST (Genova, Italy) were cultured in DMEM medium supplemented with 10% fetal bovine serum (Invitrogen), 1% penicillin/streptomycin and 2 mM L-glutamine (37 °C, 5% $CO_2$).

### 3.15. Analysis of cell viability

A549 ($5 \times 10^3$), and Hela ($5 \times 10^3$) were plated in 96-well microtiter plates and incubated for 48 h in the absence and in the presence of compounds **1**–**16** (at concentrations of 12.5, 25, 50, 100 μM). The viability of cells was determined by MTT assay to detect functional mitochondria in living cells (Mosmann, 1983), as previously reported (Altunkeyik et al., 2012). 25 μl of MTT (5 mg/mL) were added to each well and the cells were incubated for an additional 3 h. Thereafter, cells were lysed with 100 μl of a solution containing 50% (v/v) N,N-dimethylformamide and 20% (w/v) SDS (pH 4.5) to allow solubilization of dark blue crystals. The optical density (OD) of each well was measured with a microplate spectrophotometer (Mutiscan Go, Thermo Fisher Scientific Inc. Waltham, MA, USA) equipped with a 620 nm filter. $IC_{50}$ values were calculated from cell viability dose-response curves and defined as the concentration resulting in 50% inhibition of cell survival at 48 h, compared to untreated cells.

## Acknowledgements

Authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/Brazil) for fellowship to Clisiane C. S. Santos.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.phytochem.2017.04.008.

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