Revista Brasileira de Farmacognosia 27 (2017) 641–644

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Short communication

Iridoids from leaf extract of *Genipa americana*

Jovelina S.F. Alves[a], Layane A. de Medeiros[a], Matheus de F. Fernandes-Pedrosa[b], Renata M. Araújo[c], Silvana M. Zucolotto[a,*]

[a] *Laboratório de Farmacognosia, Departamento de Farmácia, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil*
[b] *Laboratório de Tecnologia e Biotecnologia Farmacêutica, Programa de Pós-graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil*
[c] *Instituto de Química, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil*

ARTICLE INFO

Article history:
Received 19 December 2016
Accepted 24 March 2017
Available online 18 August 2017

Keywords:
Genipa americana
Rubiaceae
Jenipapo
Leaves
Iridoids

ABSTRACT

Genipa americana L., Rubiaceae, is a plant native from Brazil popularly known as "jenipapo". Two iridoids, 1-hydroxy-7-(hydroxymethyl)-1,4*aH*,5*H*,7*aH*-cyclopenta[*c*]pyran-4-carbaldehyde (**1**), and iridoid 7-(hydroxymethyl)-1-methoxy-1*H*,4*aH*,5*H*,7*aH*-cyclopenta[*c*]pyran-4-carbaldehyde (**2**) were isolated and identified in the leaf extract of *G. americana*. Compounds **1** and **2** were identified for the first time in *G. americana*, and **1** has not been yet described in literature. These substances were analyzed by spectroscopic techniques such as infrared, high resolution mass spectrometry, ^{1}H and ^{13}C 1D; as well as 2D nuclear magnetic resonance. Moreover, the presence of flavonoids was detected by a preliminary analysis by Thin Layer Chromatography.

Introduction

Genipa americana L., Rubiaceae, is native plant from Brazil (Zappi, 2016) and popularly known as "jenipapo" or "jenipapeiro". It is found in Central America, South America and widely distributed in Brazil (Lorenzi and Matos, 2008). In folk medicine the extracts of leaves have been used to treat syphilis (Corrêa, 1978) and liver diseases (Agra et al., 2008).

The majority of the phytochemical and pharmacological studies were carried out with *G. americana* fruits. The chemical constituents of these fruits are mainly iridoids (Djerassi et al., 1960; Tallent, 1964; Ueda and Iwahashi, 1991; Hsua et al., 1997; Ono et al., 2005; Ono et al., 2007). These compounds are not widely distributed in the plant kingdom. Thus, they have been identified in a few families such as Apocynaceae, Loganiaceae, Lamiaceae, Scrophulariaceae and Verbenaceae (Villaseñor, 2007). Specifically to leaf extracts of *G. americana* was described the presence of geniposidic acid (Guarnaccia et al., 1972) and genipatriol (Hossain et al., 2003). Currently, only one report indicated the presence of flavonoid quercetin in fruits by HPLC analysis (Omena et al., 2012) and another work described flavonoids and tannins in its fruits by colorimetric methods (Nogueira et al., 2014).

Some pharmacological trials showed antibacterial (Tallent, 1964), antitumoral (Hsua et al., 1997), anti-inflammatory (Koo et al., 2004) and antioxidant (Omena et al., 2012) activities in the fruit extracts. The leaf extract of *G. americana* has only two studies reported. Ethanolic and hexanic extracts of that leaves showed antidiabetic effect by the inhibition of α-glucosidase enzyme ($30.44 \pm 0.10\%$ and $12.44 \pm 0.02\%$, respectively), which is compared with kojic acid, the positive control (De Souza et al., 2012). In the second study, the aqueous leaf extract showed anthelminthic activity at 100 mg/ml. The lethal concentrations (LC90) of this aqueous extract for hatching and L3 larvae development inhibition were 79.8 and 28.7 mg/ml, respectively. The extract was more effective in larval development inhibition than in hatching (Nogueira et al., 2014).

Therefore, this study aimed to perform a phytochemical study with the leaves extract of *G. americana*, since as few studies were identified in literature. According to this study, the iridoids 1-hydroxy-7-(hydroxymethyl)-1,4*aH*,5*H*,7*aH*-cyclopenta[*c*]pyran-4-carbaldehyde (**1**) and 7-(hydroximethyl)-1-methoxy-1*H*,4*αH*,5*H*,7*αH*-cyclopental[c]pyran-4-carbaldehyde **(2)** were identified for the first time in this plant, and **1** has not been yet described in literature.

Material and methods

The nuclear magnetic resonance (NMR) spectra were performed on a Bruker Avance DRX-500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer equipped with 5 mm inverse detection z-gradient probe. Chemical shifts are given in ppm relative to residual DMSO-d6 (2.5), and to the central peak of the triplet related to

* Corresponding author.
E-mail: silvanazucolotto@ufrnet.br (S.M. Zucolotto).

http://dx.doi.org/10.1016/j.bjp.2017.03.006

Table 1
^{1}H and ^{13}C NMR chemical shift compounds **1** and **2** (δ in ppm, J in Hz).

C	1		2	
	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)
1	96.89	4.95 (*d*, 5.5)	102.1	4.86 (*d*, 5.5)
3	163.2	7.58 (*s*)	162.5	7.57 (*s*)
4	123.08	–	123.7	–
5	33.2	2.98 (*q*, 8.0)	32.0	2.99 (*m*)
6	37.2	2.68 (*m*) H_α; 1.98 (*m*) H_β	37.1	2.00 (*d*, 14.7) H_α; 2.67 (*m*) H_β
7	125.54	5.65 (*s*)	126.1	5.65 (*s*)
8	144.83	–	143.6	–
9	46.8	2.53 (*m*)	45.9	2.51 (*m*)
10	59.6	3.98 (*d*, 14.8)4.09 (*d*, 14.8)	59.2	4.04 (*d*, 15.0; *d*, 13.5)
11	190.9	9.26	191.1	9.25 (*s*)
12	–	7.55 (OH) (*s*)	56.5	3.50 (OMe)

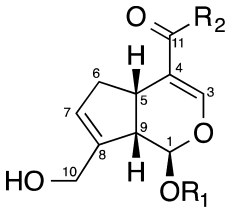
DMSO-d6 carbon (39.5 ppm). The Fourier transform infrared (FT-IR) spectra were obtained on a Perkin Elmer Spectrum 1000 spectrometer, using a universal attenuated total reflectance accessory (UATR). The high-resolution electrospray ionization mass spectra (HRESIMS) were acquired using a Shimadzu LCMS-IT-TOF (225-07100-34) spectrometer. Specific rotations were measured on a Jasco polarimeter model P-2000. HPLC preparative analysis were conducted on Shimadzu® fitted with LC-10Advp pump, UV detector Shimadzu® SPD-10AV vp, degasser (DGU-14A), and a manual injection valve 100 μl (Rheodyne®) equipped with Class Vp® version 5.0 software.

The leaves of *Genipa americana* L., Rubiaceae, were collected in May 2012 in Natal, in Brazilian state of Rio Grande do Norte (lat: – 6.1278 long: – 35.1115 WGS22). The plant material was identified by Alan de Araújo Roque (UFRN). A voucher specimen has been deposited at Herbarium/UFRN under the reference number 12251. The collection of the plant material was conducted under authorization of Brazilian Authorization and Biodiversity Information System (SISBIO) (process number 35017).

The leaves of *G. americana* (1.8 kg) were air-dried at 40 °C, powered and extracted in EtOH 70% (v/v) for 7 days (plant:solvent, 2:10, w/v), obtaining the hydroethanolic extract (HE). After that, the extract was filtered and submitted to a liquid–liquid extraction with petroleum ether (PE) (3 × 300 ml), CH_2Cl_2 (3 × 300 ml), EtOAc (3 × 300 ml), and *n*-BuOH (3 × 300 ml). The fractions were evaporated under reduced pressure (temperature below 45 °C) and yields were 37 g (PE), 12 g (CH_2Cl_2), 19 g (EtOAc), 56 g (BuOH) and 145 g (residual aqueous fraction).

In the phytochemical screening the HE and fractions were analyzed by Thin Layer Chromatography (TLC) using aluminum sheets, coated with silica gel F254 as absorbent and toluene:ethyl acetate:formic acid (5:5:0.5, v/v/v) (system I); ethyl acetate:formic acid:water:methanol (10:1.5:1.6:0.6, v/v/v/v) (system II) and ethyl acetate:formic acid:acetic acid:water (10:1.1:1.1:2.6; v/v/v/v) (system III) as mobile phases. The chromatograms were analyzed under 254 and 365 nm ultraviolet (UV) light and then sprayed with i. vanillin sulfuric acid (4%) and ii. Natural Product Reagent (0.5%)-NP Reagent. The retention factors (R_f) and colors of the spots were compared with chromatographic profiles of standards described in literature (Wagner and Bladt, 2001).

The isolation of the HE compounds from *G. americana* was started from EtOAc fraction (5 g). Thereby, this fraction was submitted to silica gel vacuum liquid column (10 × 15 cm) and eluted with CH_2Cl_2:EtOAc (50:50,40:60, 40:60, 30:70, 20:80 and 0:100; v/v) and EtOAc:MeOH (90:10, 70:30, 50:50 and 0:100; v/v). This procedure resulted in seven fractions. Fraction 3 (40 mg) (CH_2Cl_2:EtOAc, 30:70; v/v) was chromatographed further on a silica gel column (30 × 2.2 cm), eluted with $CHCl_3$:H_2O:MeOH (9:0.1:0.9; v/v; 2.0 ml min^{-1}) and 100% MeOH, affording eight fractions (FLC-1 to FLC-8). Fraction FLC-7 (40 mg) was submitted to preparative HPLC by isocratic elution: 8% MeCN in H_2O, 4.5 ml min^{-1} flow, using Waters RP 18 column (250 × 10 mm, 10 μm), and UV 254 nm, to afford **1**, 8 mg (t_R 8.8 min) and **2**, 5 mg (t_R 21.5 min).



1 R_1=R_2=H
2 R_1=CH_3; R_2=H
3 R_1=H; R_2=OCH_3

Results and discussion

The present work was conducted in order to evaluate the chemical composition of HE of *G. americana* leaves. TLC analysis of extract and fractions of *G. americana* with specific spray reagents indicated the presence of flavonoids and iridoids. Phytochemical screening (system I) of the HE and fractions, exposing with vanillin sulfuric acid, showed two brown zones (R_f = 0.9 and 0.95). These results suggest iridoids in that sample, which are common in *Genipa* fruits (Djerassi et al., 1960; Tallent, 1964; Ueda and Iwahashi, 1991; Hsua et al., 1997; Ono et al., 2005; Ono et al., 2007). Furthermore, in literature two papers reported only geniposidic acid (Guarnaccia et al., 1972) and genipatriol in the leaves (Hossain et al., 2003). TLC plate of the CH_2Cl_2 fraction was developed with system I and sprayed with NP, showing green and orange fluorescent spots (R_f = 0.46 and 0.54), suggesting flavonoids. In TLC plates analysis of the AcOEt fraction was employed the system II and to BuOH fraction, system III as the mobile phase. These plates were exposed by NP reagent, under UV 365 nm showing many yellow and orange spots (R_f = 0.43 and 0.51 and R_f = 0.22, 0.42 and 0.47), respectively. These colors imply flavonoids. In residual aqueous fraction were visualized spots with R_f and color featuring flavonoids, except brownish zone at the point of application with exposure of sulfuric vanillin, probably indicating the presence of sugars (Wagner and Bladt, 2001). As described before, most studies demonstrated iridoids in *Genipa* genus, especially to *G. americana*. However, only one report indicated the presence of flavonoid quercetin by HPLC analysis (Omena et al., 2012) and another described the presence of in its fruits by colorimetric methods (Nogueira et al., 2014).

In the phytochemical study, EtOAc (5 g) was fractionated by chromatographic procedures to afford **1** (8 mg, t_R 8.8 min) and **2** (5 mg t_R 21.5 min). Color spots observed in TLC analysis, ^{1}H and ^{13}C-NMR, ESI-MS and FT-IR confirmed the compounds **1** and **2** as iridoids (Fig. 1 and Table 1).

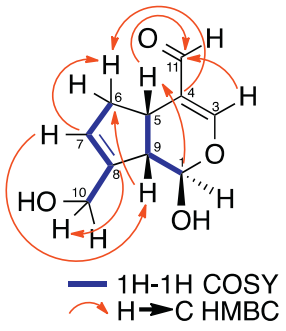


Fig. 1. Key ^{1}H–^{1}H COSY and HMBC of 1-hydroxy-7-(hydroxymethyl)-1*H*,4*aH*,5*H*,7*aH*-cyclopenta[*c*]pyran-4-carbaldehyde (**1**).

1-Hydroxy-7-(hydroxymethyl)-1*H*,4*aH*,5*H*,7*aH*-cyclopenta[*c*]pyran-4-carbaldehyde **(1)** $[\alpha]_D^{20}$ −80.4° (c 0.013, $CHCl_3$) was obtained as a brown solid and showed the molecular formula $C_{10}H_{12}O_4$ by HR-ESI-MS, based on the *quasi*-molecular ion at *m*/*z* 197.0795 $[M+H]^+$ (calcd. for $C_{10}H_{12}O_4$ *m*/*z* 197.0808), indicating five degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra (Table 1) showed mostly single peaks, suggesting compound **1** as an iridoid monomer. The IR spectrum displayed the hydroxy (3300 cm^{-1}) and carbonyl (1670 cm^{-1}) groups. The ^{1}H NMR spectrum showed two olefinic protons at δ_H 7.58 (H-3, s) and δ_H 5.65 (H-7, s); two methylene protons δ_H 1.98 (H-α-6, m) and δ_H 1.98 (H-α-6, m); two methine protons δ_H 2.98 (H-5, *q*, *J* = 8.0 Hz) and δ_H 2.53 (H-9, m); one oxymethylene δ_H 3.98 (H-10, d, *J* = 14.8 Hz); one dioxymethylene δ_H 4.95 (H-1, d, *J* = 5.5 Hz) and one aldehyde proton at δ_H 9.26 (H-11) assignable to an iridoid framework (Joubouhi et al., 2015; Lee et al., 2016). These data were confirmed by the ^{13}C NMR spectrum, which exhibited ten carbon signals, including to carbonyl carbon δ_C 190.9 (C-11); one hemiacetal carbon δ_C 163.02 (C-1); four olefinic carbons δ_C 163.2 (C-3), δ_C 123.08 (C-4), δ_C 125.54 (C-7) and δ_C 144.83 (C-8); two methylenes δ_C 37.2 (C-6) and δ_C 59.06 (C-10); and two methines 33.2 (C-5) and δ_C 46.08 (C-9). These signals were attributed after comparison with the HSQC and DEPT 135 spectra data.

The ^{1}H NMR spectrum of **1** exhibited a singlet at δ_H 9.26 and correlations with the carbon signal ($^{1}J_{CH}$) at δ_C 190.9 (C-11) in the HSQC spectrum, profiling a conjugated aldehyde group, giving support by the 1670 cm^{-1} absorption in the FTIR spectrum and representing one of the unsaturations. More two unsaturation were related for two trisubstituted vinyl group from the typical ^{13}C NMR signals at δ_C 163.3 (CH-3), 123.1 (C-4), 125.6 (CH-7) and 144.8 (C-8) as well as ^{1}H NMR signals at δ_H 7.58 (H-3, sl) and 5.65 (H-7, sl) (Rao and Chary, 2013). The ^{1}H NMR spectrum of (**1**) exhibited a singlet at δ_H 7.55 associated with a hydroxyl group, confirmed by the 3300 cm^{-1} absorption in the FTIR spectrum; even more it showed two doublets with geminal coupling at δ_H 3.98 and 4.09 (2H-10). These signals inferred this structure (Zeng et al., 2007).

In the COSY spectrum was possible to characterize the sequence of these hydrogens and confirm diastereotopic protons at δ_H 3.98 and 4.09 (2H-10) in the molecule (Fig. 1). NMR data together with the additional data spectral (Table 1) and literature data were possible to establish an iridoid nucleus for compound **1** (Zeng et al., 2007; Rao and Chary, 2013; Lee et al., 2016). This structure was confirmed by correlations shown in the HMBC spectrum for protons at δ_H 9.26 (H-11) with the carbons at δ_C 33.2 (C-5) and 163.2 (C-3); at δ_H 2.98 (H-5) with the carbons at δ_C 97.0 (C-1), 125.5 (C-7), 163.2 (C-3) and 190.9 (C-11); at δ_H 5.65 (H-7) with the carbons at δ_C 46.8 (C-9) and 59.6 (C-10). Other key HMBC correlations are shown in Fig. 1. Based on literature and biosynthetic grounds, this iridoid shows the same stereochemistry for the ring fused cis 5S:9S and for C-1-R, indicate by the negative optical rotation (Chaudhuri et al., 1979; Dinda et al., 2007a,b). The data set obtained for this compound allowed the elucidation as 1-hydroxy-7-(hydroxymethyl)-1*H*,4*aH*,5*H*,7*aH*-cyclopenta[*c*]pyran-4-carbaldehyde **(1)**, iridoid not described in literature before.

Iridoids are very reported in the *Genipa* genus, especially genipin in fruits (**3**) (Djerassi et al., 1960; Dewick, 2002). Based on these data, the structure **1** was proposed to be a new genipin derivative, with a reduction in the carbonyl group C-11, compatible with the biogenesis proposed by Dewick in 2002 for iridoids.

The compound **2** $[\alpha]_D^{20}$ −22.2° (c 0.05, $CHCl_3$), exhibited spectroscopic data very similar to compound **1**, every ^{1}H and ^{13}C NMR signals were similar except for an additional signal at δ_C 56.5 in the compound **2** that demonstrated correlation with hydrogen at δ_H 3.50 in the HSQC spectrum, indicating a methoxyl group in **2** (Table 1). Based on these findings and comparing with the literature values, the compound **2** was characterized as the iridoid garedenal-I, isolated previously from *Gardenia jasminoides* Ellis fruits (Rao and Chary, 2013). Detailed data of compounds **1** and **2** are as follow (Fig. 1). However, they are the first time reported at *G. americana*.

Iridoids are valuable for pharmaceutical applications due to various bioactive properties. Phytochemical studies showed the pharmacological potential of iridoid nucleus molecules in numerous trials, mainly anti-inflammatory, antimicrobial, antiviral, anticancer and hypoglycemic activities (Hsua et al., 1997; Koo et al., 2004; Hanh et al., 2016; Milella et al., 2016). Additionally, the iridoids may be responsible for plant defense by insect relations (Lohaus and Schwerdtfeger, 2014), and they are precursors of monoterpenoid indole alkaloids, such as vincristine and vinblastine used in the treatment of cancer (Van Moerkerckea et al., 2015). Herbal drugs commercialized as *Harpagophytum procumbens* cointain iridoids as chemical markers (Mncwangi et al., 2012). Genipin is used as crosslinking in nanotechnological formulations and other applications (Li et al., 2015). Thus, molecules of this class assemble medicinal, biological, and commercial value.

1-Hydroxy-7-(hydroxymethyl)-1*H*,4*aH*,5*H*,7*aH*-cyclopenta[*c*]pyran-4 carbaldehyde (**1**): brown solid; Brown spot R_f 0.52 after spraying the plate vanillin sulfuric acid; $CHCl_3$:H_2O:MeOH, (9:0.1:0.9; v/v/v); $[\alpha]_D^{20}$ −80.4°; IR (KBr) ν_{max}/cm^{-1} 3350, 2930, 2870, 1670, 1600, 1100. ^{1}H NMR (300 MHz, DMSOd) and ^{13}C NMR (75 MHz, DMSOd) see Table 1. Positive HRESIMS (positive mode) *m*/*z*, calcd. for $C_{10}H_{12}O_4$ $[M+H]^+$: 197.0795, found: 197.0808.

7-(Hydroxymethyl)-1-methoxy-1*H*,4*aH*,5*H*,7*aH*-cyclopenta[*c*]pyran-4-carbaldehyde (**2**): brown solid; Brown spot R_f 0.62 after spraying the plate vanillin sulfuric acid, $CHCl_3$:H_2O:MeOH (9:0.1:0.9; v/v/v); $[\alpha]_D^{20}$ −22.2° (c 0.05, $CHCl_3$). ^{1}H NMR (500 MHz, DMSOd) and ^{13}C NMR (1255 MHz, DMSOd) see Table 1.

Conclusion

In *G. americana* leaves was possible identified two iridoids: 1-hydroxy-7-(hydroxymethyl)-1,4*aH*,5*H*,7*aH*-cyclopenta[*c*]pyran-4 -carbaldehyde (**1**) and 7-(hydroxymethyl)-1-methoxy-1*H*,4*aH*, 5*H*,7*aH*-cyclopenta[*c*]pyran-4-carbaldehyde (**2**). Observing, thus, the presence of flavonoids in the leaves extract and fractions by TLC. It is so important to continue the phytochemical study to identify more secondary metabolites and investigate new biological applications for this extract and these molecules.

Author's contribution

JSFA, LAM and SMZL carried out the phytochemical process for isolation. JSFA wrote the manuscript. SMZL and RMA also contributed in the writing the manuscript. RMA performed NMR analysis. SMZL, RMA and MFP contributed to the critical reading the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

The authors acknowledge all contributors for their valuable time and commitment to the study. We also thank to the CAPES for the Masters scholarship; CNPq for the financial support (478661/2010-0) and UFRN – Universidade Federal do Rio Grande do Norte.

References

Agra, M.F., Silva, K.N., Basílio, I.J.L.D., de Freitas, P.F., Barbosa-Filho, J.M., 2008. Survey of medicinal plants used in the region Northeast of Brazil. Rev. Bras. Farmacogn. 18, 472–508.

Chaudhuri, K.R., Afifi-Yazar, F.Ü., Sticher, O., 1979. The configuration of naturally occurring iridoid glucosides at C(6) and C(8): a complementary assignment aid by ^{13}C-NMR spectroscopy. Helv. Chim. Acta 62, 1603–1604.

Corrêa, M.P., 1978. Dicionário das plantas úteis do Brasil e das exóticas cultivadas. Imprensa Nacional, Rio de Janeiro.

De Souza, P.M., De Salesi, P.M., Simeoni, L.A., Silva, E.C., Silveira, D., Magalhães, P.O., 2012. Inhibitory activity of α-amylase and α-glucosidase by plant extracts from the Brazilian cerrado. Planta Med. 78, 393–399.

Dewick, P.M., 2002. Medicinal Natural Products. John Wiley & Sons, West Sussex, England.

Dinda, B., Debnath, S., Harigaya, S., 2007a. Naturally occurring iridoids. A review, Part 1. Chem. Pharm. Bull. 55, 159–222.

Dinda, B., Debnath, S., Harigaya, S., 2007b. Naturally occurring secoiridoids and bioactivity of naturally occurring iridoids and secoiridoids. A review, Part 2. Chem. Pharm. Bull. 55, 689–728.

Djerassi, C., Gray, J.D., Kincl, F., 1960. Isolation and characterization of genipin. J. Org. Chem. 25, 2174–2177.

Guarnaccia, R., Madyastha, K.M., Tegtmeyer, E., Coscia, C.J., 1972. Geniposidic acid, an iridoid glucoside from *Genipa americana*. Tetrahedron Lett. 50, 5125–5127.

Hossain, C.F., Jacob, M.R., Clark, A.M., Walker, L.A., Nagle, D.G., 2003. Genipatriol, a new cycloartane triterpene from *Genipa spruceana*. J. Nat. Prod. 66, 398–400.

Hanh, N.P., Phan, N.H.T., Thuan, N.T.D., Hanh, T.T.H., Vien, L.T., Thao, N.P., Thanh, N.V., Cuong, N.X., Binh, N.Q., Nam, N.H., Kiem, P.V., Kim, H.O., Minh, C.V., 2016. Two new simple iridoids from the ant-plant *Myrmecodia tuberose* and their antimicrobial effects. Nat. Prod. Res. 30, 18.

Hsua, H., Yang, J., Lin, S., Linb, C., 1997. Comparisons of geniposidic acid and geniposide on antitumor and radioprotection after sublethal irradiation. Cancer Lett. 113, 31–37.

Joubouhi, C., Mabou, F.D., Tebou, P.L.F., Ngnokam, D., Harakat, D., Voutquenne-Nazabadioko, L., 2015. Five new iridoïd dimers from the fruits of *Canthium subcordatum* DC (syn, *Psydrax subcordata* DC). Phytochem. Lett. 13, 348–354.

Koo, H., Song, Y.S., Kim, H., Lee, Y., Hong, S., Kim, S., Kim, B., Jin, C., Lim, C., Park, E., 2004. Antiinflammatory effects of genipin, an active principle of gardenia. Eur. J. Pharmacol. 495, 201–208.

Lee, S.R., Clardy, J., Senger, D.R., Cao, S., Kim, K.H., 2016. Iridoid and phenylethanoid glycosides from the aerial part of *Barleria lupulina*. Rev. Bras. Farmacogn. 26, 281–284.

Li, Q., Wang, X., Lou, X., Yuan, H., Tu, H., Li, B., Zhang, Y., 2015. Genipin-crosslinked electrospun chitosan nanofibers: determination of crosslinking conditions and evaluation of cytocompatibility. Carbohydr. Polym. 130, 166–174.

Lohaus, G., Schwerdtfeger, M., 2014. Comparison of sugars, iridoid glycosides and amino acids in nectar and phloem sap of *Maurandya barclayana*, *Lophospermum erubescens*, and *Brassica napus*. Plos One 9, e87689.

Lorenzi, H., Matos, F.J.A., 2008. Plantas medicinais no Brasil: nativas e exóticas. Nova Odessa, São Paulo.

Milella, L., Milazzo, S., De Leo, M., Saltos, M.B.V., Faraone, I., Tuccinardi, T., Lapillo, M., De Tommasi, N., Braca, A., 2016. α-Glucosidase and α-amylase inhibitors from *Arcytophyllum thymifolium*. J. Nat. Prod. 79, 2104–2112.

Mncwangi, N., Chen, W., Vermaak, I., Viljoen, A.M., Gericke, N., 2012. Devil's Claw – a review of the ethnobotany, phytochemistry and biological activity of *Harpagophytum procumbens*. J. Ethnopharmacol. 143, 755–771.

Nogueira, F.A., Nery, P.S., Morais-Costa, F., Oliveira, N.J.F., Martins, E.R., Duarte, E.R., 2014. Efficacy of aqueous extracts of *Genipa americana* L. (Rubiaceae) in inhibiting larval development and eclosion of gastrointestinal nematodes of sheep. J. Appl. Anim. Res. 42, 356–360.

Omena, C.M.B., Valentim, I.V., Guedes, G.S., Rabelo, L.A., Mano, C.M., Bechara, E.J.H., Sawaya, A.C.H.F., Trevisan, M.T.S., Da Costa, J.G., Ferreira, R.C.S., Sant'ana, A.E.G., Goulart, M.O.F., 2012. Antioxidant, anti-acetylcholinesterase and cytotoxic activities of ethanol extracts of peel, pulp and seeds of exotic Brazilian fruits. Food Res. Int. 49, 334–344.

Ono, M., Ueno, M., Masouka, C., Ikeda, T., Nohara, T., 2005. Iridoid glucosides from the fruit of *Genipa americana*. Chem. Pharm. Bull. 53, 1342–1344.

Ono, M., Ishimatsu, N., Masuoka, C., Yoshimitsu, H., Tsuchihashi, R., Okawa, M., Kinjo, J., Ikeda, T., Nohara, T., 2007. Three new monoterpenoids from the fruit of *Genipa americana*. Chem. Pharm. Bull. 55, 632–634.

Rao, A.S., Chary, J.S., Merugu, R., 2013. Iridoids from *Gardenia jasminoides* Ellis. Int. J. ChemTech Res. 5, 418–421.

Tallent, W.H., 1964. Two new antibiotic cyclopentanoid monoterpenes of plant origin. Tetrahedron Lett. 20, 1781–1787.

Ueda, S., Iwahashi, Y., 1991. Production of anti-tumor-promoting iridoid glucosides in *Genipa americana* and its cell cultures. J. Nat. Prod. 54, 1677–1680.

Van Moerkerckea, A., Steensmac, P., Schweizera, F., Polliera, J., Gariboldic, I., Payned, R., Bosschea, R.V., Miettinena, K., Espozc, J., Purnamac, P.C., Kellnerd, F., Seppänen-Laaksoe, T., O'Connord, S.E., Rischere, H., Memelinkc, J., Goossensa, A., 2015. The bHLH transcription factor BIS1 controls the iridoid branch of the monoterpenoid indole alkaloid pathway in *Catharanthus roseus*. Proc. Natl. Acad. Sci. U. S. A. 112, 8130–8135.

Villaseñor, I.M., 2007. Bioactivities of iridoids. Anti-inflam. Anti-Allerg. Agents Med. Chem. 6, 307–314.

Wagner, H., Bladt, S., 2001. Plant Drug Analysis: A Thin Layer Chromatography Atlas. Springer, Germany.

Zappi, D., 2016. Genipa in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro, http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB14045 (accessed 18.01.16).

Zeng, B.Y., Mei, W.L., Zhao, Y.X., Zhuang, L., Hong, K., Dai, H.F., 2007. Two new epimeric pairs of iridoid from mangrove plant *Scyphiphora hydrophyllacea*. Chin. Chem. Lett. 19, 1509–1511.