http://dx.doi.org/10.21577/0103-5053.20190269

J. Braz. Chem. Soc., Vol. 31, No. 5, 1030-1042, 2020
Printed in Brazil - ©2020 Sociedade Brasileira de Química

Article

3-Aminofurostane Alkaloids from *Solanum paniculatum* ("Jurubeba Verdadeira") Roots

Tania M. S. Silva,[a] *Telma M. G. Silva,*[a] *Maria F. Agra*[b] *and Celso A. Camara*[a]

[a]*Laboratório de Bioprospecção Fitoquímica, Departamento de Química, Universidade Federal Rural de Pernambuco, 52171-900 Recife-PE, Brazil*

[b]*Laboratório de Taxonomia e Farmacobotânica, Departamento de Biotecnologia, Universidade Federal da Paraíba, 58051-970 João Pessoa-PB, Brazil*

The roots of *Solanum paniculatum* (Solanaceae) have extensively been used in folk medicine to treat liver infections and as a diuretic. Ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS/MS) were used for the profiling and structural characterization of alkaloids from the roots of *S. paniculatum*. Sixteen 3-aminofurostane alkaloids were characterized as novel compounds. In this study, three principal alkaloids were isolated in mixture, and their structures were established by different spectroscopic methods, including 1D, 2D nuclear magnetic resonance (NMR) experiments, and the high-resolution electrospray ionization (HR-ESI)-MS analysis. The isolated alkaloids were used to explore fragmentation pathways. Compound identification was based on the exact mass and fragmentation behaviors. Two compounds were identified as new natural compounds as: (25*R*)-3β-amino-furost-5-en-22α,26-diol *O*(26)-β-D-glucopyranoside (fatimagraine) and (25*R*)-3β-amino-furost-22-en,26-ol *O*(26)-β-D-glucopyranoside (bhattacharyyaine). The unambiguous assignments of ^{1}H and ^{13}C NMR data and chemical correction of the structure alkaloid jurubine are reported for the first time.

Keywords: *Solanum paniculatum*, jurubeba, 3-aminofurostane alkaloids, UPLC-QTOF-MS

Introduction

Solanum paniculatum (Solanaceae) is popularly known in Brazil as "jurubeba verdadeira", and "jurubeba roxa".[1] This species is exclusive to South America, found in Brazil, Paraguay, and Argentina.[2] It has a wide distribution in Brazil, occurring in all regions of the country.[3] The decoction of the roots is used in folk medicine for liver infections and as a diuretic.[1,4]

Previous phytochemical studies of *S. paniculatum* roots resulted in the identification of paniculidine;[5] (22*R*,25*S*)-3β-amino-5α-spirostan;[6] jurubine (25*S*)-3β-amino-5α-furostane-22α,26-diol *O*(26)-β-D-glucopyranoside;[7] isojuripidine, isojurubidine, and isopaniculidine.[8] From aerial parts and leaves were isolated steroidal saponins.[9-11]

As part of our chemical and pharmacological studies of Brazilian *Solanum*,[12-24] we report the chemical investigation on *S. paniculatum*. The present study with *S. paniculatum* roots resulted in the characterization of 17 3-aminofurostane alkaloids by ultra-performance liquid chromatography coupled with time-of-flight mass spectrometry (UPLC-TOF-MS/MS). Compounds **11**, **12**, and **17** were isolated, and their structures were established by different spectroscopic methods including 1D nuclear magnetic resonance (NMR) (^{1}H, attached proton test (APT)), 2D NMR experiments (correlation spectroscopy (COSY), heteronuclear single quantum correlation (HSQC), and heteronuclear multiple bond correlation (HMBC)), and the high-resolution electrospray ionization (HR-ESI)-MS analysis. Compounds **11** and **17** were identified as new natural compounds and named (25*R*)-3β-amino-furost-5-en-22α,26-diol *O*(26)-β-D-glucopyranoside (fatimagraine) and (25*R*)-3β-amino-furost-22-en-26-ol *O*(26)-β-D-glucopyranoside (bhattacharyyaine), respectively.

Experimental

General experimental procedures

NMR spectra were obtained using Bruker DRX 500

*e-mail: tania.sarmento@ufrpe.br, sarmentosilva@gmail.com
This article is part of the Special Issue Brazilian Women in Chemistry.

(500 MHz for ^{1}H and 125 MHz for ^{13}C) and Bruker DPX300 (300 MHz for ^{1}H and 75 MHz for ^{13}C) spectrometers (Karlsruhe, Germany). Samples were prepared as solutions in CD_3OD, and tetramethylsilane (TMS) was used as an internal reference. Thin layer chromatography (TLC) was performed with pre-coated silica gel 60 PF254 plates (0.25 mm, Merck, Darmstadt, Germany). A Strata Giga Tubes (SPE C18-E 70 g/150 mL, Phenomenex Inc., Torrance, USA) cartridge was employed to obtain alkaloids (Phenomenex Co., Torrance, CA, USA). Acetonitrile was obtained from Sigma (St. Louis, MO, USA). Milli-Q water (Billerica, USA) was used for the UPLC-QTOF-MS (ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry) analysis. The XEVO-G2XSQTOF mass spectrometer (Waters, Manchester, UK) was connected to the ACQUITY UPLC system (Waters, Milford, MA, USA) via an electrospray ionization interface (ESI). The chromatographic separation of compounds was performed on the ACQUITY UPLC with a conditioned autosampler at 4 °C using the Waters ACQUITY UPLC BEH C18 (2.1 × 50 mm, 1.7 µm) (Waters, Milford, MA, USA). The mobile phase consisting of water with 0.1% formic acid in water (solvent A) and acetonitrile 0.1% formic acid (solvent B) was pumped at a flow rate of 0.4 mL min^{-1}. The gradient elution program was as follows: 0**-**5 min, 5**-**10% B; 5**-**9 min, 10**-**95% B. The injection volume was 5**-**10 µL. The MS analysis was performed on a Xevo G2 QTOF (Waters MS Technologies, Manchester, UK), a quadrupole time-of-flight tandem mass spectrometer coupled with an electrospray ionization source in positive ion mode. The scan range was from *m/z* 50 to 1200 for data acquisition. In addition, MS^E experiments were carried, which allows both precursor and product ion data to be acquired in one injection. The source conditions were as follows: 3 kV capillary voltage; 120 °C source temperature; 450 °C desolvation temperature; 50 L h^{-1} cone gas flow rate; 800 L h^{-1} desolvation gas (N_2) flow rate, and 40 V cone voltage. All analyses were performed using the lockspray, which ensured accuracy and reproducibility. Leucine-enkephalin (200 pg mL^{-1}) was used as a standard or reference compound to calibrate mass spectrometers during the analyses. All data acquisition and analyses were controlled using the Waters MassLynx v. 4.1 software.

Plant material

The roots of *Solanum paniculatum* were collected in Pernambuco State, Brazil, in the municipality of Recife in December 2012. The voucher specimen (53790) has been deposited at the Herbarium Vasconcelos Sobrinho of the Universidade Federal Rural de Pernambuco (UFRPE).

Extraction and isolation

S. paniculatum dried and powdered root (981.0 g) was extracted by maceration with ethanol:NH_4OH 2% (9:1) at room temperature. After filtration, the combined extractive solutions were concentrated under reduced pressure to yield the crude extract (91.0 g). The ethanolic extract (50.0 g) was suspended with acetic acid 10% and filtered through a bed of Celite. The aqueous acid filtrate was basified with NH_4OH and left standing overnight. This solution was extracted with butanol and concentrated to produce 12.8 g of the BuOH fraction. This fraction (5.6 g) was subjected to SPE C-18 with H_2O, MeOH, and ethyl acetate as binary mixtures of increasing polarity, and yielded 62 fractions. Fractions 19-21 were eluted with 80% MeOH, and 35-42 with 100% MeOH resulting in the isolation of the compounds mixture **11/12** (7.8 mg), and **12/17** (43.7 mg).

Results and Discussion

Identification of isolated compounds

The alkaloidal fraction was subjected to UPLC-QTOF-MS^E and was used for the profiling and structural characterization of alkaloids from the roots of *S. paniculatum*. Seventeen 3-aminofurostane alkaloids were characterized and three principal alkaloids were isolated in mixture. The isolated alkaloids were used to explore fragmentation pathways. The structures of compounds **11**, **12**, and **17** were identified by the combined use of 1D NMR (^{1}H and ^{13}C NMR), 2D-NMR (^{1}H-^{1}H COSY, HSQC, HMBC), and MS spectra. The unambiguous assignments of ^{1}H and ^{13}C NMR data of compound **12** (jurubine) are reported for the first time and involved a combination of homo- and heteronuclear 2D NMR techniques (Table 1). Both compounds **11** and **17** were isolated in the mixture with jurubine, and all showed a positive Dragendorff reagent test, indicating the alkaloidal nature of the structures.

Compound **12** exhibited a protonated molecular ion at *m/z* 596.4156 ($[M + H]^+$, calcd. 596.4156 for $C_{33}H_{58}NO_8$) in the (+)-HRESIMS. The positive ESI-MS spectra of compound **12** suggested an aminofurostane structure with one glucopyranose. Compounds **12** and **15** are isomers with the same molecular weight and same ions fragments. The MS/MS experiment (Table 2) showed a prominent fragment ion at *m/z* 399.3271 corresponding to the loss of glucosyl, water, and ammonia $[M + H - H_2O - NH_3 - glucose]^+$. The hydroxyl at C-20 was readily eliminated as water to produce the ion at *m/z* 578.4054 $[M + H - H_2O]^+$; the amino group at C-3 was confirmed by the subsequent loss of ammonia to generate the ion at *m/z* 561.3792 $[M + H - H_2O - NH_3]^+$.

Table 1. ^{1}H and ^{13}C NMR data (500 and 125 MHz, CD_3OD) for compound **12** (jurubine)

	δ_C / ppm	δ_H / ppm (mult., *J* in Hz)	$^{2}J_{CH}$	$^{3}J_{CH}$
1	36.9	1.50 (m), 1.21 (m)	2H-2	3H-19
2	30.0	1.72 (m), 1.12 (m)		
3	52.0	2.70 (m)		
4	38.7	1.46 (m), 1.03 (m)	H-9	2H-6
5	46.9	1.17 (m)		3H-19
6	31.5	1.75 (m), 1.38 (m)	2H-7	
7	32.8	1.70 (m), 0.97 (m)	2H-6	
8	36.6	1.58 (m)	2H-7, H-9	
9	55.9	0.71 (m)		3H-19
10	38.2		3H-19	
11	22.2	1.53 (m), 1.38 (m)	H-9	
12	41.2	1.70 (m), 1.11 (m)		3H-18
13	42.3		2H-12, 3H-18	H-20, H-16, 2H-15
14	57.9	1.23 (m)		3H-18
15	32.2	1.95 (ddd, *J* 12.0, 10.0, 5.0 Hz) 1.24 (m)	H-14, H-16	H-17
16	82.6	4.35 (q, *J* 7.0 Hz)	2H-15, H-17	
17	65.3	1.70 (m)	H-16	3H-18, 3H-21, H-20
18	17.2	0.79 (s)	H-17	H-20
19	12.9	0.85 (s)		2H-1, H-9
20	41.3	2.15 (q, *J* 7.0 Hz)	3H-21	H-16, 2H-23
21	16.2	0.99 (d, *J* 7.0 Hz)		
22	116.0		H-20, 2H-23	3H-21, 2H-24
23	31.5	1.65 (m), 1.82 (m)	2H-24	H-20, H-25
24	29.1	1.15 (m), 1.62 (m)	H-25	3H-27
25	35.1	1.75 (m)	2H-26, 2H-24, 3H-27	
26	76.1	3.72 (dd, *J* 10.0, 7.0 Hz), 3.37 (dd, *J* 10.0, 7.0 Hz)	H-25	2H-24, H-1'
27	17.6	0.94 (d, *J* 7.0 Hz)		2H-24, 2H-26
Glucose				
1'	104.7	4.23 (d, *J* 8.0 Hz)	H-2'	H-3', 2H-26
2'	75.3	3.18 (dd, *J* 9.0, 8.0 Hz)	H-1', H-3'	H-4'
3'	78.3	3.31 (dd, *J* 9.0, 8.0 Hz)	H-2'	H-1', H-5'
4'	71.9	3.27 (m)	H-3', H-5'	2H-6'
5'	78.1	3.24 (m)	H-4', 2H-6'	H-3'
6'	62.9	3.85 (dd, *J* 12.0, 2.0 Hz), 3.65 (dd, *J* 12.0, 5.0 Hz)	H-5'	

The ion at *m/z* 416.3552 $[M + H - H_2O - glucose]^+$ corresponded to the loss of glucose. From the fragment ion at *m/z* 399.3271 with a proton added to the oxygen of C-16 and a positive charge in this oxygen, the bond of C-16–O was broken, and the double electrons were transferred to the oxygen. Due to the presence of the enol, tautomerization was produced at the carbonyl of C-22.[25] The loss of one water molecule produced the ion at *m/z* 381.3143, and finally, the losses of C_6H_8 (–96 Da) and $C_8H_{14}O$ (–126 Da) provided the ion at *m/z* 285.3136 and 255.2118, respectively. The proposed fragmentation pathways of compound **12** are shown in Figure 1. The ^{1}H NMR (Table 1) spectrum of the aglycone moiety of compound **12** showed signals of four steroid methyl groups at δ 0.79 (s, H-18), 0.85 (s, H-19),

Table 2. Characterization of 3-aminofurostane alkaloids from *S. paniculatum* roots by UPLC-QTOF-MS^E

Compound	t_R / min	$[M + H]^+$ (*m/z*)	$[M + H]^+$ (*m/z*) calculated	MS/MS fragments (*m/z*) Tentative identification
1	2.84	626.3898	626.3898	3-amino-dihydroxy-dehydro-furostane-22-ol 608.3860 $[M + H - H_2O]^+$, 591.2657 $[M + H - H_2O - NH_3]^+$, 573.3530 $[M + H - 2H_2O - NH_3]^+$, 464.3369 $[M + H - glucose]^+$, 429.3031 $[M + H - H_2O - NH_3 - glucose]^+$, 411.2908 $[M + H - 2H_2O - NH_3 - glucose]^+$, 393.2814 $[M + H - 3H_2O - NH_3 - glucose]^+$, 269.1812 $[M + H - 3H_2O - NH_3 - glucose - C_8H_{12}O]^+$
2	2.99	772.4477	772.4477	3-amino-dihydroxy-dehydro-furostane-22-ol-26-di-glucopyranoside 754.4370 $[M + H - H_2O]^+$, 610.3963 $[M + H - glucose]^+$, 593.3848 $[M + H - NH_3 - glucose]^+$, 431.3193 $[M + H - NH_3 - 2glucose]^+$, 413.3193 $[M + H - H_2O - 2glucose - NH_3]^+$, 395.2957 $[M + H - 2H_2O - 2glucose - NH_3]^+$, 377.2877 $[M + H - 3H_2O - 2glucose - NH_3]^+$, 281.2274 $[M + H - 3H_2O - 2glucose - NH_3 - C_6H_8O]^+$, 271.2069 $[M + H - H_2O - 2glucose - NH_3 - C_8H_{14}O_2]^+$, 253.1966 $[M + H - 3H_2O - 2glucose - NH_3 - C_8H_{12}O]^+$
3	3.11	774.4634	774.4634	3-amino-dihydroxy-furostane-22-ol-26-di-glucopyranoside 756.4547 $[M + H - H_2O]^+$, 738.4454 $[M + H - 2H_2O]^+$, 594.4022 $[M + H - H_2O - glucose]^+$, 432.3488 $[M + H - H_2O - 2glucose]^+$, 415.3056 $[M + H - H_2O - 2glucose - NH_3]^+$, 397.3122 $[M + H - 2H_2O - 2glucose - NH_3]^+$, 379.2999 $[M + H - 3H_2O - 2glucose - NH_3]^+$, 283.2423 $[M + H - 3H_2O - 2glucose - NH_3 - C_6H_8O]^+$, 353.1966 $[M + H - 3H_2O - 2glucose - NH_3 - C_8H_{14}O]^+$
4	3.22	628.4055	628.4055	3-amino-dihydroxy-furostane-22-ol 610.3965 $[M + H - H_2O]^+$, 593.3704 $[M + H - H_2O - NH_3]^+$, 575.3593 $[M + H - H_2O - NH_3]^+$, 448.3441 $[M + H - H_2O - glucose]^+$, 431.3163 $[M + H - H_2O - NH_3 - glucose]^+$, 413.3058 $[M + H - 2H_2O - NH_3 - glucose]^+$, 395.2949 $[M + H - 3H_2O - NH_3 - glucose]^+$, 377.2850 $[M + H - 4H_2O - NH_3 - glucose]^+$, 281.2280 $[M + H - 4H_2O - NH_3 - glucose - C_6H_8O]^+$, 269.2440 $[M + H - 2H_2O - NH_3 - glucose - C_8H_{14}O_2]^+$, 251.1802 $[M + H - 4H_2O - NH_3 - glucose - C_8H_{14}O]^+$
5	3.29	774.7634	774.7634	3-amino-dihydroxy-furostane-22-ol-26-di-glucopyranoside 756.4543 $[M + H - H_2O]^+$, 738.4458 $[M + H - 2H_2O]^+$, 594.4016 $[M + H - H_2O - glucose]^+$, 432.2822 $[M + H - H_2O - 2glucose]^+$, 415.3064 $[M + H - H_2O - 2glucose - NH_3]^+$, 397.3122 $[M + H - 2H_2O - 2glucose - NH_3]^+$, 379.2999 $[M + H - 3H_2O - 2glucose - NH_3]^+$, 283.2423 $[M + H - 3H_2O - 2glucose - NH_3 - C_6H_8O]^+$, 353.1966 $[M + H - 3H_2O - 2glucose - NH_3 - C_8H_{14}O]^+$
6	3.58	612.4106	612.4106	3-amino-hydroxy-furostane-22-ol 594.4011 $[M + H - H_2O]^+$, 577.3746 $[M + H - H_2O - NH_3]^+$, 559.3644 $[M + H - 2H_2O - NH_3]^+$, 432.3482 $[M + H - H_2O - glucose]^+$, 415.3210 $[M + H - H_2O - NH_3 - glucose]^+$, 397.3113 $[M + H - 2H_2O - NH_3 - glucose]^+$, 379.3657 $[M + H - 3H_2O - NH_3 - glucose]^+$, 383.2972 $[M + H - 2H_2O - NH_3 - glucose - C_6H_8O]^+$, 271.2612 $[M + H - 2H_2O - NH_3 - glucose - C_8H_{16}O_2]^+$, 253.2489 $[M + H - 2H_2O - NH_3 - glucose - C_8H_{14}O]^+$
7	3.90	772.4477	772.4477	3-amino-dihydroxy-dehydro-furostane-22-ol-26-di-glucopyranoside isomer 754.4370 $[M + H - H_2O]^+$, 610.3963 $[M + H - glucose]^+$, 593.3848 $[M + H - NH_3 - glucose]^+$, 431.3193 $[M + H - NH_3 - 2glucose]^+$, 413.3193 $[M + H - H_2O - 2glucose - NH_3]^+$, 395.2957 $[M + H - 2H_2O - 2glucose - NH_3]^+$, 377.2877 $[M + H - 3H_2O - 2glucose - NH_3]^+$, 281.2274 $[M + H - 3H_2O - 2glucose - NH_3 - C_6H_8O]^+$, 271.2069 $[M + H - H_2O - 2glucose - NH_3 - C_8H_{14}O_2]^+$, 253.1966 $[M + H - 3H_2O - 2glucose - NH_3 - C_8H_{12}O]^+$
8	4.12	612.4106	612.4106	3-amino-hydroxy-furostane-22-ol isomer 594.4010 $[M + H - H_2O]^+$, 577.3746 $[M + H - H_2O - NH_3]^+$, 559.3651 $[M + H - 2H_2O - NH_3]^+$, 432.3492 $[M + H - H_2O - glucose]^+$, 415.3207 $[M + H - H_2O - NH_3 - glucose]^+$, 397.3108 $[M + H - 2H_2O - NH_3 - glucose]^+$, 379.3657 $[M + H - 3H_2O - NH_3 - glucose]^+$, 383.2972 $[M + H - 2H_2O - NH_3 - glucose - C_6H_8O]^+$, 271.2612 $[M + H - 2H_2O - NH_3 - glucose - C_8H_{16}O_2]^+$, 253.2489 $[M + H - 2H_2O - NH_3 - glucose - C_8H_{14}O]^+$
9	4.21	758.4689	758.4685	3-amino-furostane-22-ol-26-di-glucopyranoside 740.4604 $[M + H - H_2O]^+$, 578.4096 $[M + H - H_2O - glucose]^+$, 417.3568 $[M + H - NH_3 - 2glucose]^+$, 416.3518 $[M + H - H_2O - 2glucose]^+$, 399.3269 $[M + H - H_2O - NH_3 - 2glucose]^+$, 285.2586 $[M + H - H_2O - NH_3 - 2glucose - C_6H_8O]^+$, 381.3177 $[M + H - 2H_2O - NH_3 - 2glucose]^+$, 255.2120 $[M + H - 2H_2O - NH_3 - 2glucose - C_8H_{14}O]^+$

Table 2. Characterization of 3-aminofurostane alkaloids from *S. paniculatum* roots by UPLC-QTOF-MS^E (cont.)

Compound	t_R / min	$[M + H]^+$ (*m/z*)	$[M + H]^+$ (*m/z*) calculated	MS/MS fragments (*m/z*) Tentative identification
10	4.28	610.3948	610.3949	3-amino-hydroxy-dehydro-furostane-22-ol 592.3856 $[M + H - H_2O]^+$, 574.3755 $[M + H - 2H_2O]^+$, 430.3345 $[M + H - H_2O$ – glucose$]^+$, 413.3070 $[M + H - H_2O - NH_3$ – glucose$]^+$, 395.2962 $[M + H - 2H_2O - NH_3$ – glucose$]^+$, 377.2246 $[M + H - 3H_2O - NH_3$ – glucose$]^+$, 269.2612 $[M + H - 2H_2O - NH_3$ – glucose – $C_8H_{16}O_2]^+$, 251.1813 $[M + H - 2H_2O - NH_3$ – glucose – $C_8H_{14}O]^+$
11	4.54	594.4001	594.4000	(25*R*)-3β-amino-furost-5-en-22α,26-diol *O*(26)-β-D-glucopyranoside (fatimagraine) 576.3913 $[M + H - H_2O]^+$, 559.3636 $[M + H - H_2O - NH_3]^+$, 414.3391 $[M + H - H_2O$ – glucose$]^+$, 397.3105 $[M + H - H_2O - NH_3$ – glucose$]^+$, 379.3011 $[M + H - 2H_2O - NH_3$ – glucose$]^+$, 283.2433 $[M + H - 2H_2O - NH_3$ – glucose – $C_6H_8O]^+$, 253.1956 $[M + H - 2H_2O - NH_3$ – glucose – $C_6H_{16}O_2]^+$
12	4.63	596.4156	596.4156	(25*R*)-3β-amino-5α-furostane-22α,26-diol *O*(26)-β-D-glucopyranoside (jurubine) 578.4058 $[M + H - H_2O]^+$, 561.3786 $[M + H - H_2O - NH_3]^+$, 416.3538 $[M + H - H_2O$ – glucose$]^+$, 399.3956 $[M + H - H_2O - NH_3$ – glucose$]^+$, 381.3146 $[M + H - 2H_2O - NH_3$ – glucose$]^+$, 285.2581 $[M + H - 2H_2O - NH_3$ – glucose – $C_6H_8O]^+$, 255.2109 $[M + H - 2H_2O - NH_3$ – glucose – $C_6H_{16}O_2]^+$
13	4.79	756.4528	756.4528	3-amino-dehydro-furostane-22-ol-26-di-glucopyranoside 738.4423 $[M + H - H_2O]^+$, 594.4001 $[M + H$ – glucose$]^+$, 577.3755 $[M + H - NH_3$ – glucose$]^+$, 559.3644 $[M + H - H_2O - NH_3$ – glucose$]^+$, 432.3481 $[M + H$ – 2glucose$]^+$, 414.3373 $[M + H - H_2O$ – 2glucose$]^+$, 413.3075 $[M + H - NH_3$ – 2glucose$]^+$, 397.3119 $[M + H - H_2O - NH_3$ – glucose$]^+$, 253.1961 $[M + H - 2H_2O - NH_3$ – 2glucose – $C_8H_{14}O]^+$
14	5.08	594.4001	594.4000	fatimagraine isomer 576.3906 $[M + H - H_2O]^+$, 559.3669 $[M + H - H_2O - NH_3]^+$, 414.3371 $[M + H - H_2O$ – glucose$]^+$, 397.3110 $[M + H - H_2O - NH_3$ – glucose$]^+$, 379.3002 $[M + H - 2H_2O - NH_3$ – glucose$]^+$, 283.1955 $[M + H - 2H_2O - NH_3$ – glucose – $C_6H_8O]^+$, 253.1955 $[M + H - 2H_2O - NH_3$ – glucose – $C_6H_{16}O_2]^+$
15	5.17	596.4156	596.4156	jurubine isomer 578.4072 $[M + H - H_2O]^+$, 561.3793 $[M + H - H_2O - NH_3]^+$, 416.3518 $[M + H - H_2O$ – glucose$]^+$, 399.3274 $[M + H - H_2O - NH_3$ – glucose$]^+$, 381.3143 $[M + H - 2H_2O - NH_3$ – glucose$]^+$, 285.2583 $[M + H - 2H_2O - NH_3$ – glucose – $C_6H_8O]^+$, 255.2118 $[M + H - 2H_2O - NH_3$ – glucose – $C_6H_{16}O_2]^+$
16	6.04	576.3894	576.3894	dehydro-bhattacharyyaine 559.3630 $[M + H - NH_3]^+$, 397.3109 $[M + H - NH_3$ – glucose$]^+$, 414.2479 $[M + H$ – glucose$]^+$, 379.3002 $[M + H - H_2O - NH_3$ – glucose$]^+$, 283.2439 $[M + H - 2H_2O - NH_3$ – glucose – $C_6H_8O]^+$, 253.1955 $[M + H - 2H_2O - NH_3$ – glucose – $C_6H_{16}O_2]^+$
17	6.15	578.4051	578.4051	(25*R*)-3β-amino-furost-22-en,26-ol *O*(26)-β-D-glucopyranoside (bhattacharyyaine) 561.3789 $[M + H - NH_3]^+$, 399.3263 $[M + H - NH_3$ – glucose$]^+$, 416.3539 $[M + H$ – glucose$]^+$, 381.3161 $[M + H - H_2O - NH_3$ – glucose$]^+$, 285.2591 $[M + H - 2H_2O - NH_3$ – glucose – $C_6H_8O]^+$, 255.2121 $[M + H - 2H_2O - NH_3$ – glucose – $C_6H_{16}O_2]^+$

t_R: retention time.

0.99 (d, *J* 7.0 Hz, H-21), and 0.94 (d, *J* 7.0 Hz, H-27), two methylene protons bearing an oxygen function at δ 3.72 (dd, *J* 10.0, 7.0 Hz) and 3.37 (dd, *J* 10.0, 7.0 Hz, H-26), one methine proton bearing an oxygen function at δ 4.35 (q, *J* 7.0 Hz, H-16), and one β-amino group at δ 2.70 (m, H-3). The ^{1}H NMR spectrum (Table 1) of the sugar moiety of compound **12** showed signals of one methylene of glucopyranose (δ 3.85 (dd, *J* 12.0, 2.0 Hz) and 3.65 (dd, *J* 12.0, 5.0 Hz, H-6')). The β-configuration of D-glucose was determined by the $J^{1,2}$ value of 8.0 Hz. These results suggested that compound **12** consists of a furostane type aglycone with one amino group and one sugar moiety of a glucopyranose. From the ^{13}C NMR spectrum (Table 1), the results were further supported by the presence of signals of four methyl groups of steroid at δ 17.2 (C-18), 12.9 (C-19), 16.1 (C-21), and 17.6 (C-27), two methylene carbons bearing an oxygen function at δ 76.1 (C-26) and 62.9 (C-6'), one methine carbon bearing an oxygen function at δ 82.6 (C-16), three quaternary carbon at δ 38.2 (C-10), 42.3 (C-13), and 116.0 (C-22), one anomeric carbons of sugar at δ 104.7 (C-1'), and one signal for a carbon-bearing nitrogen at δ 52.0 (C-3). This pattern of proton

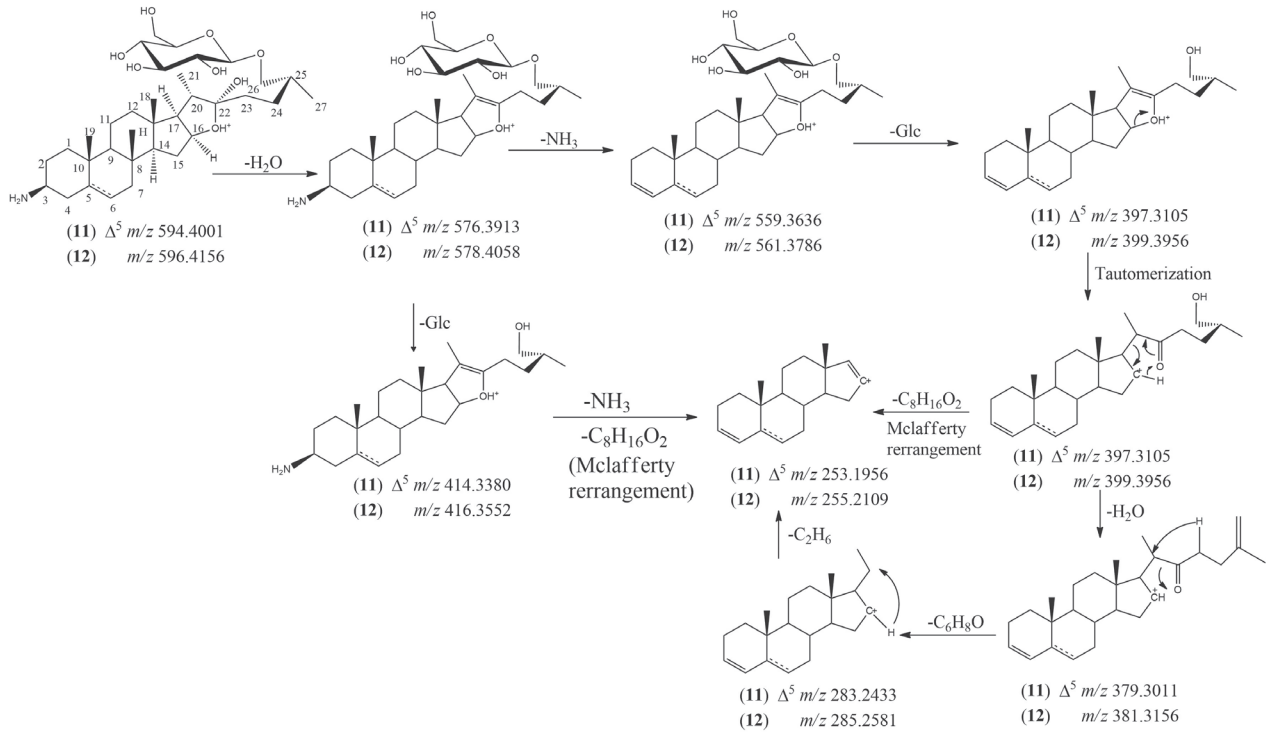

Figure 1. The proposed fragmentation pathways of fatimagraine (**11**) and jurubine (**12**) using ESI-qTOF-MS/MS.

chemical shifts, the ^{13}C NMR spectrum of compound **12** with the presence of anomeric at δ 104.7 (C-1') and the hemiacetal at δ 116.0 (C-22) carbons signal suggested the furostane nature of the aglycone of compound **12**. All the above proton resonances were unambiguously associated with relevant carbon atoms by using the HSQC spectrum. The steroid and glucoside moiety were assigned from their proton-proton correlations in the ^{1}H-^{1}H COSY spectrum, and the long-range correlations between protons and carbons by the HMBC spectrum. In particular, it was observed HMBC cross-peaks H_3-19 (δ 0.85) with C-10 (δ 38.2), C-1 (δ 36.9), C-5 (δ 46.9), and C-9 (δ 55.9); H_3-18 (δ 0.79) with C-13 (δ 42.3), C-12 (δ 41.2), and C-14 (δ 57.9); and H_2-23 (δ 1.65 and 1.82) with C-20 (δ 41.3) and the hemiacetal carbon C-22 (δ 116.0). Conversely, the HMBC cross-peak of H-26 (δ 3.72 and 3.37) with the anomeric carbon at δ 104.74 (C-1') allowed us to identify C-26 as a further glycosidic linkage site. The relative stereochemistry of compound **12** indicated the A/B *trans*-ring fusion due to the signals corresponding to carbons C-5 and C-9 at δ 46.9 and 55.9, respectively.[26] The B/C *trans* and C/D *trans*-ring fusion geometry evidences the usual furostane ring junctions with rings.[27] The 22α orientation of compound **12** was established on the basis of the ^{1}H NMR resonances of H-21 (δ 0.99) and H-16 (δ 4.35) suggesting that, most likely, they are deshielded by the *cis*-oriented OH-22 group.[28] The 25*R* configuration was determined on the basis of differences in chemical shifts of the geminal protons at H_2-26 that exhibit pronounced dependence; the difference (Δab = δa – δb) between their chemical shifts (Δab ≤ 0.48 for 25*R*; Δab ≥ 0.57 for 25*S*) seems to be of general applicability for ascertaining the 25*R*/25*S* orientation of the 27-methyl group of furostane-type steroidal saponins.[27] Consequently, based on the results described above, the structure of compound **12** was established as (25*R*)-3β-amino-5α-furostane-22α,26-diol *O*(26)-β-D-glucopyranoside. Analogously, as a result of the inspection of the ^{1}H and ^{13}C NMR spectra of compound **12** (Table 1), the same gross structure of jurubine[29] was identified. Therefore, we argue that these molecules should differ only in the stereochemistry of one chiral carbon. This was readily identified as the C-25 carbon. Compound **12** have the 25*R* configuration as shown above, and jurubine has been reported to have the 25*S* configuration.[7] Since there are no NMR data in the literature that prove the absolute stereochemistry of C-25*S* for jurubine, it is possible that the correct structure of jurubine is presented in this work, i.e., jurubine has actually the C-25*R* configuration. Jurubine was only identified in nature in two plant species: roots of *Solanum paniculatum*[7] and *Solanum torvum*.[30] The unambiguous assignments of ^{1}H and ^{13}C NMR data of jurubine (**12**) are reported for the first time in this study.

The structure of compounds **11** and **17** was readily elucidated on the basis of their considerable similarities to compound **12**. The (+)-HRESIMS spectrum of compound **11** exhibited a protonated molecular ion at

m/z 594.4001 ([M + H]$^+$, calcd. 594.4000 for $C_{33}H_{56}NO_8$); compound **17** showed a protonated molecular ion at *m/z* 578.4051 ([M + H]$^+$, calcd. 578.4051 for $C_{33}H_{56}NO_7$) in accordance with the ^{13}C-APT spectra. A good level of similarity in the chemical shifts of the glucose was observed for the two compounds **11** and **17**, however, with some differences in the aglycone. The molecular weight of compounds **11** and **17** that have mass units lesser than those of compound **12** indicates that **11** has two hydrogen atoms less, and **17** has one oxygen atom less than **12**, respectively. The MS/MS experiment of compound **11** (Table 2) showed a profile similar to that of **12**, with the difference in all ions fragments of only –2 Da due to the presence of a double bond between the C-5 and C-6 bonds of the molecule (Figure 1). The ^{1}H and ^{13}C NMR spectra of compound **11** (Table 3) indicated a 25*R*-3β-aminofurostane-type steroidal alkaloid when compared with those of compound **12**. The comparison of the whole ^{13}C NMR spectrum of **12** with that of **11** showed that a set of signals corresponding to carbons C-1, C-5, C-6, C-8, C-10, and C-19 at δ 41.1, 140.0, 124.3, 35.2, 37.9, and 19.8 showed variations in the values of chemical shifts, respectively, while all other signals remained almost unaffected. It was observed that the signals of C-5 and C-6 at δ 140.0 and 124.3, respectively, were markedly displaced downfield when compared to C-5 and C-6 of **12** at δ 46.9 and 31.5, respectively. The signal observed in the ^{1}H NMR spectrum at δ 5.35 (d, *J* 4.5 Hz, 1H) and 1.06 (s, 3H) corresponding to H-6 and Me-19, respectively, confirmed the presence of an olefinic bond between carbons 5 and 6. Compound **11** was elucidated by data from the spectra of ^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, HSQC, and HMBC (Table 3) as (25*R*)-3β-amino-furost-5-en-22α,26-diol *O*(26)-β-D-glucopyranoside and named fatimagraine.

The product ion spectrum of compound **17** (*m/z* 578.4051, [M + H]$^+$) show one ion product at *m/z* 561.3809 [M + H – NH_3]$^+$ attributable to the loss of NH_3, indicating that this compound did not present the hydroxyl group at the C-22 position because there was no loss of water molecule from the protonated molecular ion (–18 Da). The abundant ion at *m/z* 399.3260 might be obtained by the loss of glucose [M + H – NH_3 – glucose]$^+$ from *m/z* 561.3809. The subsequent loss of H_2O, C_6H_8, and $C_8H_{14}O$ furnish the ions product at *m/z* 381.3167, 285.2591, and 255.2119, respectively. Compound **16** presented the same ionization profile as **17** (Table 2), with the only difference at ions fragments of –2 Da due to the presence of a double bond between the C-5 and C-6 bonds of the molecule (Figure 2). The ^{1}H and ^{13}C NMR spectra of compound **17** (Table 4) indicated a 25*R*-3β-aminofurostane-type steroidal alkaloid when compared with those of **11** and **12**. The aglycone nature of compound **17** was also manifested by its ^{1}H and ^{13}C NMR, principally on the carbon and hydrogen values of the E-ring when compared with that in compound **12**. The unsaturation in both C-20 and C-22 showed a significant difference in the carbon values of C-16 (δ 85.8), C-20 (δ 104.8), C-21 (δ 12.0), and C-22 (δ 153.0). The unambiguous assignments of ^{1}H and ^{13}C NMR data of **17**, including 2D experiments (^{1}H-^{1}H COSY, HSQC, and HMBC) (Table 4), allowed to this compound to be identified as (25*R*)-3β-amino-furost-22-en-26-ol *O*(26)-β-D-glucopyranoside and named batthacharyyaine.

The lack of finding spirostane alkaloids type compounds in the roots of *S. paniculatum* in this study, despite of

Table 3. ^{1}H and ^{13}C NMR data (500 and 125 MHz, CD_3OD) for compound **11** (fatimagraine)

	δ_C / ppm	δ_H / ppm (mult., *J* in Hz)	$^2J_{CH}$	$^3J_{CH}$
1	41.1	1.76 (m), 1.45 (m)		3H-19
2	29.0	1.30 (m), 1.70 (m)		
3	51.8	3.31 (m)		
4	40.8	2.18 (m)	H-9	
5	140.0			3H-19
6	124.3	5.35 (d, *J* 4.5 Hz)		
7	32.8	1.94 (m), 1.28 (m)		
8	35.2	1.56 (m)		
9	51.6	0.96 (m)		3H-19
10	37.9		3H-19	
11	22.1	1.56 (m), 1.30 (m)	H-9	
12	42.2	1.70 (m), 1.13 (m)		3H-18
13	41.9		3H-18	
14	57.5	1.23 (m)		3H-18
15	33.3	1.97 (m), 1.25 (m)		H-17
16	82.5	4.36 (m)	2H-15, H-17	
17	65.9	1.75 (m)	H-16	3H-18, 3H-21
18	16.9	0.83 (s)		
19	19.8	1.06 (s)		H-9
20	41.3	2.17 (m)	3H-21	
21	16.3	0.98 (d, *J* 7.0 Hz)		H-20
22	114.0			3H-21
23	31.5	1.81 (m), 1.65 (m)		H-25
24	28.8	1.20-1.40 (m)	H-25	3H-27
25	35.1	1.72 (m)	3H-27	
26	76.1	3.73 (m) 3.35 (m)		H-1'
27	17.4	0.95 (d, *J* 7.0 Hz)		2H-24, 2H-26
Glucose				
1'	104.7	4.23 (d, *J* 8.0 Hz)	H-2'	H-3', 2H-26
2'	75.3	3.13 (m)	H-1', H-3'	H-4'
3'	78.3	3.38 (m)		H-1', H-5'
4'	71.9	3.28 (m)	H-5'	2H-6'
5'	78.0	3.26 (m)	H-4', 2H-6'	H-3'
6'	62.9	3.86 (dd, *J* 12.0, 2.0 Hz) 3.65 (m)		

Table 4. ^{1}H and ^{13}C NMR data (500 and 125 MHz, CD_3OD) for compound **17** (bhattacharyyaine)

	δ_C / ppm	δ_H / ppm (mult., *J* in Hz)	$^{2}J_{CH}$	$^{3}J_{CH}$
1	36.9	1.50 (m), 1.21 (m)	2H-2	3H-19
2	30.0	1.72 (m), 1.12 (m)		
3	52.0	2.70 (m)		
4	38.7	1.46 (m), 1.03 (m)	H-9	2H-6
5	46.9	1.17 (m)		3H-19
6	31.5	1.75 (m), 1.38 (m)	2H-7	
7	32.8	1.70 (m), 0.97 (m)	2H-6	
8	36.6	1.58 (m)	2H-7, H-9	
9	55.9	0.71 (m)		3H-19
10	38.2		3H-19	
11	22.2	1.53 (m), 1.38 (m)	H-9	
12	41.2	1.70 (m), 1.11 (m)		3H-18
13	44.7		3H-18	H-16, 2H-15
14	56.2	1.23 (m)		3H-18
15	32.2	1.95 (ddd, *J* 12.0, 10.0, 5.0 Hz), 1.65 (m)	H-14, H-16	H-17
16	85.8	4.70 (dt, *J* 8.0, 2.0 Hz)	2H-15, H-17	
17	65.3	2.44 (d, *J* 7.5 Hz)	H-16	3H-18, 3H-21
18	14.9	0.69 (s)	H-17	
19	12.9	0.85 (s)		2H-1, H-9
20	104.8		H-17	3H-21
21	12.0	1.58 (s)		
22	153.0			H-17
23	32.2	1.81 (m), 1.65 (m)		H-25
24	29.1	1.15 (m), 1.62 (m)	H-25	3H-27
25	35.1	1.75 (m)	2H-26, 2H-24, 3H-27	
26	76.1	3.72 (dd, *J* 10.0, 7.0 Hz), 3.37 (dd, *J* 10.0, 7.0 Hz)	H-25	2H-24, H-1’
27	17.6	0.94 (d, *J* 7.0 Hz)		2H-24, 2H-26
Glucose				
1’	104.7	4.23 (d, *J* 8.0 Hz)	H-2’	H-3’, 2H-26
2’	75.3	3.18 (dd, *J* 9.0, 8.0 Hz)	H-1’, H-3’	H-4’
3’	78.3	3.31 (dd, *J* 9.0, 8.0 Hz)	H-2’	H-1’, H-5’
4’	71.9	3.27 (m)	H-3’, H-5’	2H-6’
5’	78.0	3.24 (m)	H-4’, 2H-6’	H-3’
6’	62.9	3.85 (dd, *J* 12.0, 2.0 Hz), 3.65 (dd, *J* 12.0, 5.0 Hz)	H-5’	

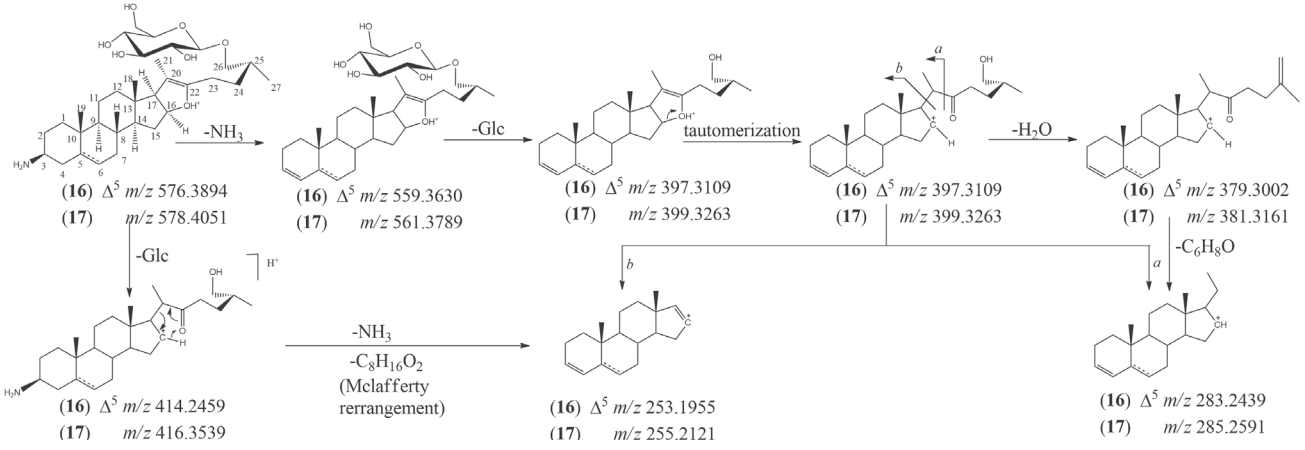

Figure 2. The proposed fragmentation pathways of **16** and battacharyyaine (**17**) using ESI-qTOF-MS/MS.

being reported in the literature,[6] may be due to the direct analysis of the alkaloidal fraction. According to Li *et al.*,[25] conventional methods present disadvantages because of the cyclization of furostanol to spirostanol aglycone, which can yield several structures that are alternatives to that of the original compound. These probable artifacts have been previously reported as natural alkaloids.[6,8]

Characterization of compounds by UPLC-QTOF-MS/MS

The UPLC-QTOF-MS^E experiments were performed to identify alkaloids in *Solanum paniculatum* roots. Only aglycone structure skeleton present in steroidal alkaloid glycoside as jurubine [3-amino-furostane-*O*(26)-β-D-glucopyranoside] (or its isomer) was detected in all compounds. The compounds exhibited the presence of unsaturation, hydroxyls, or additional glucose in the structure as variations. The common sugar moiety is D-glucose; it is linked to the aglycone through the hydroxy group typically at C-26 and C-3-linked to an amino group.

The alkaloidal fraction was subjected to UPLC-QTOF-MS^E; the profile of the base peak ion (BPI) chromatograms showed 17 main peaks (Figure 3). In ESI$^+$, alkaloids always produced a protonated ion $[M + H]^+$; the common characteristics for all compounds comprise glycosylation pattern at C-26 and furostanol-type aglycone structure.[31] The hydroxyl group was characterized by the neutral loss of 18 Da (loss of water). The presence of additional glycosyl residues was observed by the loss of 162 Da.

The evidence that all detected structures are glycoalkaloids was based on the protonated peak in the positive mode $[M + H]^+$ to the molecular ion with even mass (compounds contain one nitrogen atom). The furostane-type compounds with the 22-OH group (compounds **1**-**15**) resulted in an exclusive detection of $[M + H - H_2O]^+$ in the positive mode. Only compounds **16** and **17** with unsaturation between C-20 and C-22 did not show $[M + H - H_2O]^+$; however, they presented $[M + H - NH_3]^+$. Table 2 shows the high-resolution mass data and fragments data in the positive ion mode of the 3-aminofurostane alkaloids from *Solanum paniculatum* roots. Their analyses and comparisons with structures reported in the literature[8] showed that out of the seventeen identified glycoalkaloid, only compound **12** (jurubine) had been previously reported, all other 16 are new compounds.

Each identified glycoalkaloid showed its pair with only one additional unsaturation such as compounds $[M + H]^+$:

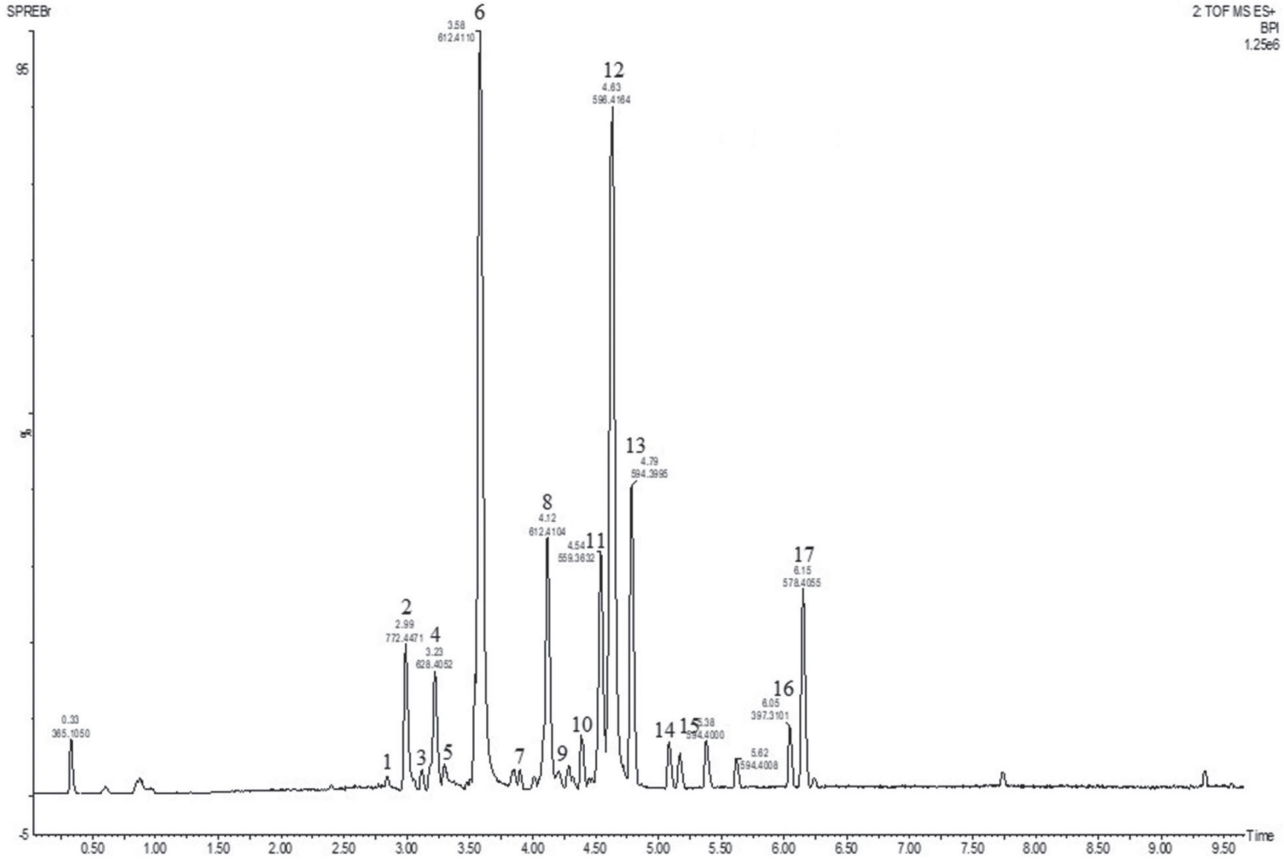

Figure 3. ESI base peak ion (BPI) chromatogram of the *Solanum paniculatum* roots analyzed by UPLC-QTOF-MS.

1/4 (*m/z* 626.3898/628.4055), **2/3** (*m/z* 772.4477/774.4634), **6/10** (*m/z* 612.4106/610.3948), **9/13** (*m/z* 758.4689/756.4528), **11/12** (*m/z* 594.4001/596.4156), and **16/17** (*m/z* 576.3894/578.4051). Furthermore compounds **2**, **7** (*m/z* 772.4477); **3**, **5** (*m/z* 774.4634); **6**, **8** (*m/z* 612.4106); **11**, **14** (*m/z* 594.4001), and **12**, **15** (*m/z* 596.4156) are isomers (Table 2).

Compounds **1**, **2**, **3**, **4**, **5**, and **7** exhibited two hydroxyl groups, which may be attached at the carbons of the A-D rings (perhydrocyclopentenophenanthrene nucleus). Likewise, compounds **6**, **8** and **10** showed only one hydroxyl group. Compounds **9** and **13** showed two more glycosides. Analyses of the mass spectra of these compounds supported the identification. The MS^2 experiment showed fragment ions characteristics of consecutive losses of water (−18 Da) and/or glucose (−162 Da). Compounds **1**-**8** and **10** eliminated at least two molecules of water whereas compounds **2**, **3**, **5**, **7**, **9** and **13** showed the loss of two glucose units. Compounds **11** and **12**, and **17** were isolated and identified by ^1H and ^{13}C NMR.

The ESI-QTOF-MS/MS investigation of the fragmentation mechanism of each 3-aminofurostane alkaloids was performed, and the characteristic fragment ion derived from the fission of aglycone with crucial fragment ions are presented in Table 2. Compounds **3**, **5**, **6**, **8**, **9**, **12**, **15**, and **17** (no unsaturation in the A-D rings) produced fragment ions at *m/z* 415/416, except compound **4** (*m/z* 413). The unsaturated compounds (A-D rings) **2**, **7**, **10**, **11**, **13**, **14**, and **16**, present characteristic fragments at *m/z* 414/413, except compound **1** (*m/z* 411). These ions

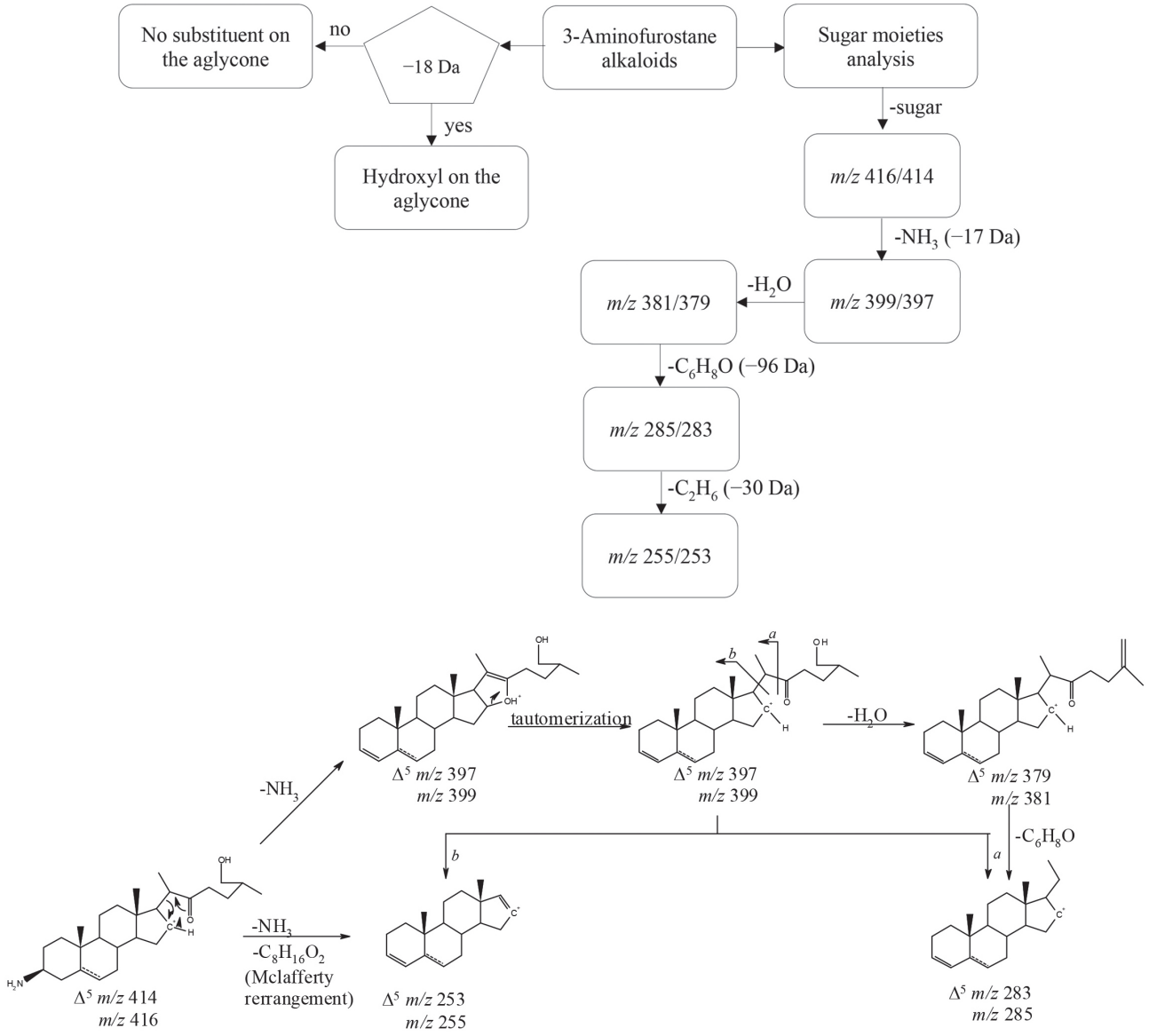

Figure 4. Flowchart for structural analysis of 3-aminofurostane alkaloids with ESI positive experiment.

are derived from the sequential loss of sugar moieties, water, and ammonia groups from the molecular weight of protonated aglycone. The predominant ions were detected at *m/z* 399/397 and 381/379 (Figures 1, 2 and 4) due to the loss of ammonia and one molecule of water from the ion product at *m/z* 416/414, respectively. At this time, a proton added to the oxygen of C-16 and leading to the formation of a positive charge on the oxygen allowed the electron pair transfer from C-16 to the oxygen and the fission of the C-16–O bond. Due to the presence of enol,

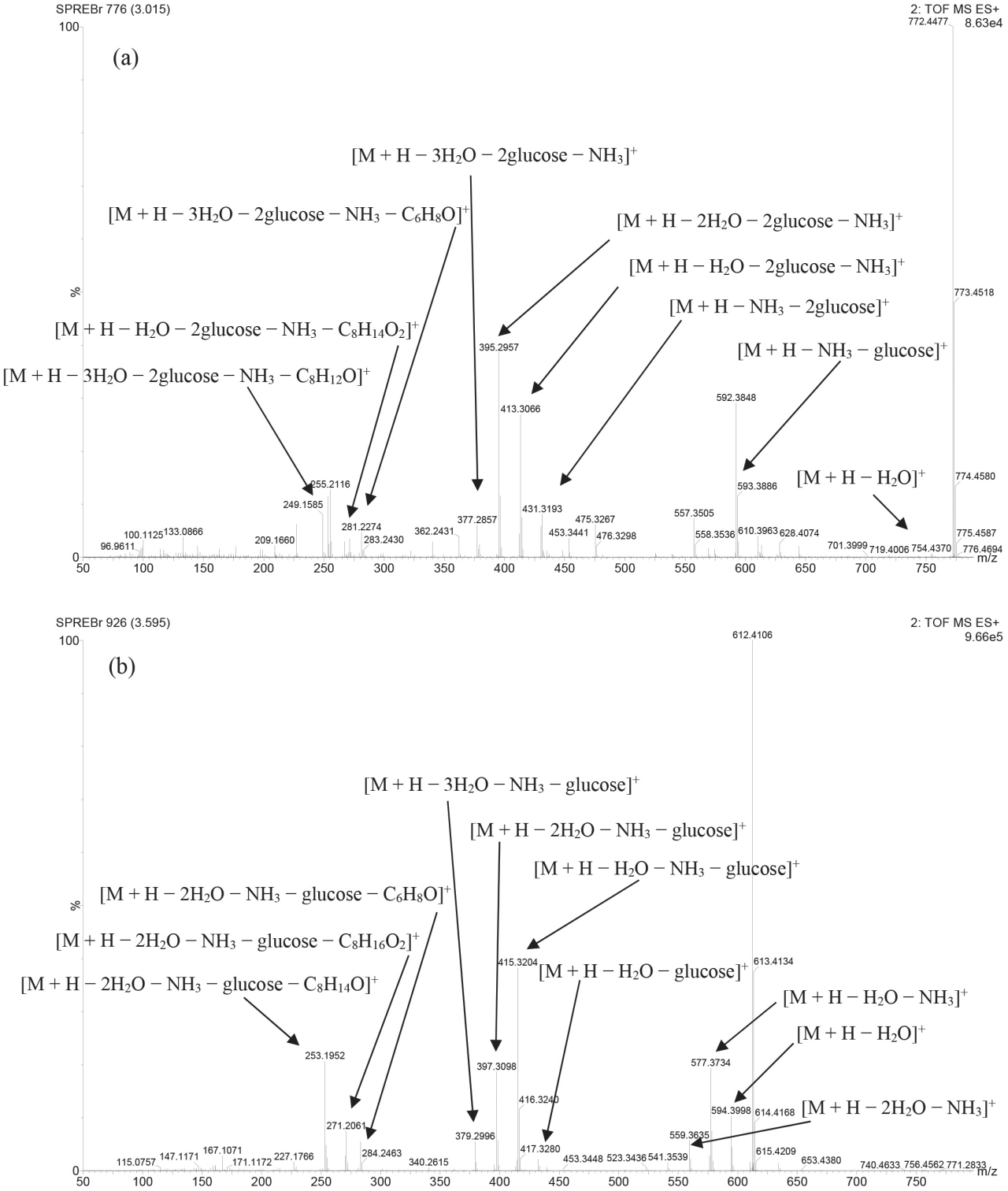

Figure 5. (a) Product ion spectrum of the ion $[M + H]^+$ *m/z* 772.4477 (**2**); (b) product ion spectrum of the ion $[M + H]^+$ *m/z* 612.4106 (**6**).

the carbonyl of C-22 was produced by the tautomerization. From this tautomer, the elimination of H_2O produced the ion at *m/z* 381/379. The ion at *m/z* 285/283 was formed by the hydrogen transfer from, C-23 to C-20, and the fission of bond C-20–C-22. The carbonyl on C-22 played the key role in the loss of $C_8H_{14}O$. The fission of bond C-17–C-20 led to the formation of the ion at *m/z* 253/255 (Figures 4 and 5). This pathway is attributable to the hydrogen transfer from C-16 to the carbonyl via the Mclafferty rearrangement. Table 2 summarizes the characteristic fragments observed in the MS/MS spectra of alkaloids from the *S. paniculatum* roots. Compounds **2** (*m/z* 772.4477) and **6** (*m/z* 612.4106) are representative of 3-amino-22-hydroxylated furostane alkaloids, with (**2**) and without (**6**) unsaturation at A-D rings, respectively. The typical MS/MS spectrum is shown in Figure 5. As an example, the fragmentation pathway proposed for **2** and **6** is a property that could be extended to other alkaloids. The scheme shown in Figure 4 summarizes the differences in fragmentation behavior that can be used for the assignment of 3-aminofurostane alkaloids according to the proposed stepwise analysis strategy of mass fragmentation spectra. The proposed fragmentation pathways of the new compounds **11**, **12**, and **16**, **17** are presented in Figures 1 and 2, respectively.

Although shown to be a powerful tool for the assignment of 3-aminofurostane alkaloids, the presented method of mass spectra interpretation cannot completely solve the structure of newly detected molecules (positions of hydroxyl and sugar residues, unsaturation in the A-D rings, and stereochemistry). The correct order of sugar residues can only be partially determined, and the exact position of substituents/double bonds on the aglycones results are not completely clear. However, such information is very helpful in discriminating between the (often) large numbers of possible structures obtained.

Conclusions

The analysis of *Solanum paniculatum* roots by UPLC-TOF-MS/MS resulted in the characterization of 17 3-aminofurostane alkaloids; out of these, only jurubine had previously been reported in the literature. The NMR analysis of the main isolated compounds allowed the identification of two new compounds: (25*R*)-3β-amino-furost-5-en-22α,26-diol *O*(26)-β-D-glucopyranoside (fatimagraine) and (25*R*)-3β-amino-furost-22-en-26-ol *O*(26)-β-D-glucopyranoside (bhattacharyyaine). The unambiguous assignments of ^1H and ^{13}C NMR data of jurubine are reported for the first time with rectification of the structure data.

Supplementary Information

The NMR and MS (**11**, **12**, and **17**) spectra of isolated compounds are shown in the Supplementary Information, available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

This study was financially supported by grants from CNPq, FACEPE (grant No. PRONEM APQ-0741106/2014), and CAPES. The authors are also thankful to the CENAPESQ for the use of facilities.

References

1. Agra, M. F.; Baracho, G. S.; Nurit, K.; Basílio, I. J. L. D.; Coelho, V. P. M.; *J. Ethnopharmacol.* **2007**, *111*, 383.
2. Nee, M. In *Solanaceae IV: Advances in Biology and Utilization*; Nee, M.; Symon, D. E.; Lester, R. N.; Jessop, J., eds.; Royal Botanic Garden: Kew, UK, 1991, 257.
3. Agra, M. F.; Silva, K. N.; Raquel, L.; *Acta Bot. Bras.* **2009**, *23*, 826.
4. http://portalarquivos2.saude.gov.br/images/pdf/2017/setembro/11/Monografia-Solanum-paniculatum.pdf, accessed in November 2019.
5. Meyer, K.; Bernoulli, F.; *Pharm. Acta Helv.* **1961**, *36*, 80.
6. Schreiber, K.; Ripperger, H.; Budzikiewicz, H.; *Tetrahedron Lett.* **1965**, *6*, 3999.
7. Schreiber, K.; Ripperger, H.; *Tetrahedron Lett.* **1966**, *7*, 5997.
8. Cambiaghi, S.; Dradi, E.; Longo, R.; *Ann. Chim. (Rome, Italy)* **1971**, *61*, 99.
9. Valerino-Díaz, A. B.; Gamiotea-Turro, D.; Zanatta, A. C.; Vilegas, W.; Martins, C. H. G.; Silva, T. S.; Rastrelli, L.; dos Santos, L. C.; *J. Agric. Food Chem.* **2018**, *66*, 8703.
10. Valadares, Y. M.; Brandão, G. C.; Kroon, E. G.; Souza-Filho, J. D.; Oliveira, A. B.; Braga, F. C.; *Z. Naturforsch., C: J. Biosci.* **2009**, *64*, 813.
11. Vieira-Júnior, G. M.; da Rocha, C. Q.; Rodrigues, T. S.; Hiruma-Lima, C. A.; Vilegas, W.; *Food Chem.* **2015**, *186*, 160.
12. Clementino-Neto, J.; Pereira, J. C.; Vasconcelos, L. H. C.; de Souza, I. L. L.; Silva, A. D. S.; Silva, T. M. G.; Ramos, N. S. M.; Pessoa, H. L. F.; Silva, T. M. S.; da Silva, B. A.; de Cavalcante, F. A.; *Planta Med.* **2016**, *82*, 58.
13. Cornelius, M. T. F.; de Carvalho, M. G.; da Silva, T. M. S.; Alves, C. C. F.; Siston, A. P. N.; Alves, K. Z.; Sant'Anna, C. M. R.; Neto, M. B.; Eberlin, M. N.; Braz-Filho, R.; *J. Braz. Chem. Soc.* **2010**, *21*, 2211.
14. Magalhães, F. S.; Cassiano, D. S. A.; Branco, C. R. C.; Silva, T. R. S.; Lins, A. C. S.; Silva, T. M. S.; Branco, A.; *Free Radicals Antioxid.* **2014**, *4*, 15.

15. Rios, R.; Silva, H. B. F.; Carneiro, N. V. Q.; Pires, A. O.; Carneiro, T. C. B.; Costa, R. S.; Marques, C. R.; Machado, M. S. S.; Velozo, E. S.; Silva, T. M. G.; Silva, T. M. S.; Conceição, A. S.; Alcântara-Neves, N. M.; Figueiredo, C. A.; *J. Ethnopharmacol.* **2017**, *209*, 32.
16. Silva, T. M. S.; Braz-Filho, R.; de Carvalho, M. G.; Agra, M. F.; *Biochem. Syst. Ecol.* **2002**, *30*, 479.
17. Silva, T. M. S.; Braz-Filho, R.; de Carvalho, M. G.; Agra, M. F.; *Biochem. Syst. Ecol.* **2002**, *30*, 1083.
18. Silva, T. M. S.; Nascimento, R. J. B.; Camara, C. A.; Castro, R. N.; Braz-Filho, R.; Agra, M. D. F.; de Carvalho, M. G.; *Biochem. Syst. Ecol.* **2004**, *32*, 513.
19. Silva, T. M. S.; Costa, R. A.; Oliveira, E. J.; Barbosa-Filho, J. M.; Agra, M. F.; Camara, C. A.; *J. Braz. Chem. Soc.* **2005**, *16*, 1467.
20. Silva, T. M. S.; Batista, M. M.; Camara, C. A.; Agra, M. F.; *Ann. Trop. Med. Parasitol.* **2005**, *99*, 419.
21. Silva, T. M. S.; Camara, C. A.; Agra, M. F.; de Carvalho, M. G.; Frana, M. T.; Brandoline, S. V. P. B.; Paschoal, L. S.; Braz-Filho, R.; *Fitoterapia* **2006**, *77*, 449.
22. Silva, T. M. S.; Nascimento, R. J. B.; Batista, M. M.; Agra, M. F.; Camara, C. A.; *Braz. J. Pharmacogn.* **2007**, *26*, 35.
23. Silva, T. M. S.; Camara, C. A.; Freire, K. R. L.; da Silva, T. G.; Agra, M. F.; Bhattacharyya, J.; *J. Braz. Chem. Soc.* **2008**, *19*, 1048.
24. Silva, T. M. S.; de Carvalho, M. G.; Braz-Filho, R.; *Quim. Nova* **2009**, *25*, 375.
25. Li, R.; Zhou, Y.; Wu, Z.; Ding, L.; *J. Mass Spectrom.* **2006**, *41*, 1.
26. da Silva, B. P.; Parente, J. P.; *Z. Naturforsch., B: J. Chem. Sci.* **2008**, *63*, 95.
27. Agrawal, P. K.; *Steroids* **2005**, *70*, 715.
28. Dini, I.; Tenore, G. C.; Trimarco, E.; Dini, A.; *Food Chem.* **2005**, *93*, 205.
29. Vieira, P. M.; Marinho, L. P. M.; Ferri, S. C. S.; Chen-Chen, L.; *An. Acad. Bras. Cienc.* **2013**, *85*, 553.
30. Schreiber, K.; Ripperger, H.; *Kulturpflanze* **1968**, *15*, 199.
31. Heinig, U.; Aharoni, A. In *Plant Isoprenoids: Methods and Protocols, Methods in Molecular Biology*, vol. 1153; Rodríguez-Concepción, M., ed.; Springer: New York, USA, 2014, p. 171-185.

Submitted: July 12, 2019
Published online: December 10, 2019