Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
21(4): 615-621, Jul./Aug. 2011

Evaluation of the bactericidal and trypanocidal activities of triterpenes isolated from the leaves, stems, and flowers of *Lychnophora pinaster*

Viviane G. C. Abreu,[1] Jacqueline A. Takahashi,[1] Lucienir P. Duarte,[1] Dorila Piló-Veloso,[1] Policarpo A. S. Junior,[2] Rosana O. Alves,[2] Alvaro J. Romanha,[2] Antônio F. C. Alcântara*[,1]

[1]*Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, Brazil,*
[2]*Laboratório de Parasitologia Celular e Molecular, Centro de Pesquisas René Rachou-Fiocruz, Brazil.*

Abstract: The phytochemical investigation on the aereal parts of *Lychnophora pinaster* Mart., Asteraceae, was carried to isolation of triterpenes. 3-*O*-Acetyl-lupeol (**1**), 3-*O*-acetyl-pseudotaraxasterol (**2**), and 3-*O*-acetyl-α-amyrin (**3**) were isolated from hexanic extract and 4,4-dimethyl-cholesta-22,24-dien-5-ol (**4**), α-amyrin (**5**), and lupeol (**6**) were isolated from hexanic/dichlorometanic extract of the leaves. Compounds Δ^7-bauerenyl acetate (**7**), friedelin (**8**), stigmasterol (**9**), and sitosterol (**10**) were isolated from the hexanic/dichlorometanic extract of the stems. The steroids **9** and **10** were also isolated from the hexanic/dichlorometanic extract of the flowers. Triterpenes **1, 3, 4**, and **7** are described for the first time in the genus *Lychnophora*. The apolar fractions of the leaf and stem extracts and some isolated triterpenes showed low trypanocidal activity. Moreover, apolar fractions of the leaf and stem extracts and **5** showed antibacterial action against *Staphylococcus aureus*.

Article

Received 13 Oct 2010
Accepted 2 Feb 2011
Available online 3 Jun 2011

Keywords:
Lychnophora pinaster
triterpenes
bactericidal activity
trypanocidal activity

ISSN 0102-695X
doi: 10.1590/S0102-695X2011005000095

Introduction

The Asteraceae family comprises about 23000 species distributed in 1600 genera (Gao et al., 2010). The genus *Lychnophora* (Asteraceae) is endemic in the central region of Brazil, mainly in rupestral fields in the states of Minas Gerais, Goiás, and Bahia (Robinson, 1999). This genus comprises 68 species (Mansanares et al., 2002) and some of them are listed as species threatened with extinction (Silveira et al., 2005). *Lychnophora* species are popularly used to treat wounds, bruise, pain, rheumatism, and inflammation (Borsato et al., 2000). *Lychnophora* species also show antitumoral (Merten et al., 2006), antimicrobial (Saúde et al., 2002), anti-pyretic, analgesic (Ferraz Filha et al., 2006), antioxidant, anticonvulsivant (Taleb-Contini et al., 2008), and trypanocidal (Grael et al., 2005) properties. Their biological activities have been attributed to sesquiterpene lactones, lignans, and caffeoylquinic acid derivatives isolated from polar extracts of the leaves and roots (Santos et al., 2005). The trypanocidal activity of *Lychnophora* species has been attributed to flavanoids, sesquiterpene lactones, and caffeoylquinic acids isolated from their alcoholic extracts (Takeara et al., 2003).

Lychnophora pinaster Mart., Asteraceae, is popularly used as analgesic, anti-rheumatic, and trypanocidal agents (Silveira et al., 2005). This species also shows significant antinociceptive and anti-inflammatory activities (Guzzo et al., 2008). Rutin, quercetin, isochlorogenic acid, caffeic acid, isovitexin, vitexin, sesquiterpene derivatives (Alcântara et al., 2005; Leite et al., 2008), and the triterpenes lupeol, a mixture of α- and β-amyrin, and friedelin (Silveira et al., 2005) were previously isolated from its aerial parts. Although several studies have lately related triterpenes to treatment of the trypanosomiases (Rosas et al., 2007; Leite et al., 2006; Cunha et al., 2006) and bactericidal activity (Al-Fatimi et al., 2010), no data are available about the trypanocidal and bactericidal activities of triterpenes isolated from *Lychnophora* species. The present work describes an investigation of the chemistry of leaves, stems, and flowers of *L. pinaster* for triterpenes, which were tested for antibacterial and trypanocidal activities.

Materials and Methods

General procedures

Uncorrected melting points were determined using Mettler equipment, model FP80 SNR H22439. The IR spectra were taken on a Perkin Elmer - *Spectrum One* (ATR) spectrometer. The ^{1}H and ^{13}C NMR spectra as well as the ^{1}H-^{1}H COSY, ^{1}H-^{1}H NOESY, ^{1}H-^{13}C HMBC, ^{1}H-^{13}C HMQC, and ^{1}H-^{13}C HSQC experiments were performed on a Bruker DRX400 AVANCE spectrometer, using $CDCl_3$ as solvent. The chemical shifts were measured in parts per million (δ) relative to TMS, which was used as an internal standard. The coupling constants (*J*) were recorded in Hertz. Mass spectra (GC-EI-MS) were obtained using a Varian 2800 gas chromatograph coupled to Varian Saturn 4000 electron impact mass spectrometer. The chromatographic separations were obtained using 100% dimethylpolysiloxane HP-1 column (50 m x 0.25 mm *i.d.*, 0.20 mm film) under the following conditions: column temperature programmed from 150 °C (isothermal for 7 min) to 300 °C at the rate of 40 °C/min, maintaining final temperature for 40 min. The mobile phase was H_2 (1.0 mL/min). The temperature of the injector and detector were 250 °C and 300 °C, respectively. An aliquot (3.0 µL) of sample solution (10 µg/100 µL) was injected and desorption in the GC injector was carried out in the split mode (1:15). The detector voltage was 300 V, the electron impact ionization potential was 70 eV, the acquisition rate was twenty spectra/s, and the mass range was 30 to 500 (*m/z*). The compounds were identified through comparison of the fragmentation profile of mass spectrum of the sample with the correspondent available in the NIST (2005) standard mass fragmentation data bank.

Plant material

Lychnophora pinaster Mart., Asteraceae, was collected in April 2007 at Moeda Mountain, in Nova Lima city, Minas Gerais State (Brazil). A voucher specimen of *L. pinaster* was deposited in the *herbarium* of the Instituto de Ciências Biológicas of the Universidade Federal de Minas Gerais, under code BHCB: 24322.

Extraction and isolation of constituents

Leaves, stems, and flowers of *L. pinaster* were previously separated, dried at 60 °C until constant weight was achieved (about one week), and finally powdered. The samples were named L (5698.0 g), S (4604.0 g), and F (710.0 g), respectively. All samples were submitted to EtOH extraction for a week, providing the corresponding extracts LE (625.16 g), SE (144.37 g), and FE (79.48 g) after solvent evaporation. LE, SE, and FE were submitted to chromatographic column (CC) using silica gel as the stationary phase (CCS) and elution with hexane, CH_2Cl_2, and EtOAc in increasing polarity order. Fractions of LE eluted with hexane (94.92 g) were again submitted to CCS eluted with hexane and CH_2Cl_2 in increasing polarity order, successively providing a mixture of **1** and **2** (5.14 g) and compound **3** recrystallized from MeOH (1.19 g). The LE fractions eluted with hexane/CH_2Cl_2 (1:1) (LE-2; 7.59 g) were again submitted to CCS eluted with hexane and CH_2Cl_2 in increasing polarity order. The LE-2 fractions eluted with hexane/CH_2Cl_2 (4:1) were recrystallized from MeOH, providing **4** (0.009 g). The LE-2 fractions eluted with hexane/CH_2Cl_2 (3:1) were recrystallized from MeOH, providing **5** (1.250 g). The LE-2 fractions eluted with hexane/CH_2Cl_2 (1:1) were recrystallized from MeOH, providing a mixture of **5** and **6** (1.139 g).

The SE fractions eluted with hexane/CH_2Cl_2 (9:1) provided a white solid, which was recrystallized from acetone (SE-1; 0.126 g). SE-1 was submitted to CC using alumina as stationary phase and eluted with hexane and CH_2Cl_2 in increasing polarity order. The fractions eluted with hexane provided **7** (0.051 g). The SE-1 fractions eluted with hexane/CH_2Cl_2 (7:3) provided **8** (0.035 g). The SE fractions eluted with hexane/CH_2Cl_2 (4:1) were washed with petroleum ether and recrystallized from EtOH, providing a mixture of **5** and **6** (0.041 g). The SE fractions eluted with hexane/CH_2Cl_2 (1:1) were dissolved in a mixture of MeOH and activated charcoal, and filtered to remove chlorophyll. The white solid was submitted to CCS eluted with hexane and CH_2Cl_2, in increasing polarity order. The fractions eluted with CH_2Cl_2 were recrystallized from EtOH, providing a mixture of **9** and **10** in a ratio of 2.5:1.0 (0.340 g). The FE fractions eluted with hexane/CH_2Cl_2 (1:1) (7.231 g) underwent the same procedure for chlorophyll extraction described above. The white solid was submitted to CCS eluted with hexane and CH_2Cl_2 in increasing polarity order. The fractions eluted with hexane/CH_2Cl_2 (1:1) were recrystallized from MeOH, providing a mixture of **9** and **10** in a ratio of 2.5:1.0 (1.165 g).

Antibacterial tests

Bioassays were conducted with Gram-positive (*Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 11779, and *Listeria monocytogenes* ATCC 15313) and Gram-negative (*Escherichia coli* ATCC 25723, *Salmonella typhimurium* ATCC 14028, and *Citrobacter freundi* ATCC 8090) bacteria. In the agar diffusion test (Takahashi et al., 2006), microorganisms were individually inoculated in vials containing Brain

Heart Infusion broth (BHI) and subsequently incubated in an oven at 37 °C for 18 h. An aliquot of this material was transferred to a tube containing solutions of NaCl and $MgSO_4.7H_2O$ to prepare the inoculum. Petri dishes containing culture medium (#1 Antibiotic Broth) and inoculum were prepared. Sterile 6 mm diameter filter paper discs were impregnated with 1 mL of each extract (100 µg/mL). Disks containing each sample were placed on the Petri dishes with the aid of sterile tweezers. A disk containing the positive control (disk impregnated with antibiotic chloramphenicol) or negative control (disk containing the solvent used to dissolve the sample) was placed at the center of each disk. The inhibition zones were read after 24 h of incubation.

In the minimum inhibitory concentration (MIC) test (Lana et al., 2006), microorganisms were inoculated into test tubes containing BHI broth and incubated in an oven at 37 °C for 18 h. Then, this suspension was transferred to a tube containing sterile saline solution to reach a suspension (inoculum) compatible with the McFarland scale 5. To assay each sample, test tubes containing BHI culture medium were used. Samples dissolved in DMSO were placed in the test tubes. The inoculum was added to each test tube. The tubes were incubated in an oven at 35 °C for 18 h. Readings were done after 18 h of incubation. MIC value was assigned to the tube containing the smallest dilution that did not present turbidity, and thus was the lowest concentration that inhibited the growth of the test microorganism.

Trypanocidal test

The *in vitro* assay with *T. cruzi* blood stream forms was carried out using blood from Swiss albino mice collected in the parasitaemia peak (7th day) after infection with the Y strain of *T. cruzi* (Oliveira et al., 2006). The infected blood was diluted with normal murine blood and RPMI 1640 medium 1:2 (pH 7.2-7.4) to the concentration of 2.0 x 10^6 trypomastigotes/mL. Solutions of each sample were prepared in DMSO and added to infected blood in duplicate to the wells of a 96 microwell plate providing a final drug concentration of 500 µg/mL (1.2-3.0 mM). To reproduce the blood bank conditions, plates were incubated at 4 °C for 24 h. The experiments were repeated two times. Afterwards, the parasite concentration was evaluated using an optical microscope with 400x magnification. DMSO and gentian violet were used as negative and positive controls, respectively. DMSO was not added to the positive control. The trypanocidal activity was expressed as percent reduction of the parasite number (lysis) comparing the wells with drugs with those without drugs.

Results and Discussion

Identification of the isolated compounds

The mixture of **1** and **2** showed strong IR absorptions at 1733, 1244, and 1174 cm^{-1}, which are characteristic of ester groups. The ^{1}H NMR spectrum showed signals at δ_H 5.36-5.12 and 4.69-4.45 attributed to alkenylic and carbinolic hydrogen atoms, respectively. The other hydrogen signals are registered between δ_H 2.0 and 0.8, characteristic of hydrogen atoms of pentacyclic triterpenes. The ^{13}C NMR showed signals at δ_C 174.0, 171.0, 81.3, and 80.9, which were assigned to two ester groups (Olea & Roque, 1990). Signals at δ_C 151.2 and 109.6 are characteristic of lupeol derivatives and signals at δ_C 139.8 and 118.9 are characteristic of pseudotaraxasterol derivatives. The ^{13}C NMR data of the mixture of **1** and **2** are in agreement with the corresponding data described for 3-*O*-acetyl-lupeol and 3-*O*-acetyl-pseudotaraxasterol, respectively (Mahato & Kundu, 1994).

The IR spectrum of **3** was similar to that of **1** and **2**, also indicating the structure of an acetylated triterpene. The mass spectrum showed the molecular ion peak at *m/z* 468, which corresponds to $C_{32}H_{52}O_2$. The ^{1}H NMR spectrum showed signals at δ_H 5.16 and 4.51, attributed to alkenyl and carbinolic hydrogen atoms, respectively. The other hydrogen signals are registered between δ_H 2.0 and 0.8. The ^{13}C NMR spectrum showed signals at δ_C 171.4, 139.6, and 124.3, which are characteristic of acetylated triterpene with ursan skeleton. The HSQC contour map showed correlations between singlet signal at δ_H 2.05 with the carbon signal at δ_C 21.3, characteristic of an acetyl group. The signal at δ_H 2.05 correlates with carbon signal at δ_C 81.0 and signal at δ_H 4.51 correlates with carbon signal at δ_C 171.4, by HMBC experiments, indicating the position of the acetyl group at C-3. The ^{13}C NMR data of **3** are in agreement with the corresponding data described for 3-*O*-acetyl-α-amyrin (Mahato & Kundu, 1994).

Compound **4** was only identified by GC/MS analysis. The GC chromatogram of **4** showed a peak (*TR* = 54.942 min; molecular ion peak: *m/z* 412) with mass spectrum similar to that of 4,4-dimethyl-cholesta-22,24-dien-5-ol (Zhuang et al., 2010), according to the NIST data bank.

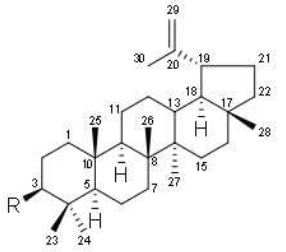
The IR spectrum of **5** showed absorptions at 3276 and 1036 cm^{-1}, which are characteristic of hydroxyl groups. The ^{1}H NMR spectrum showed signals at δ_H 5.17 and 4.61, which are attributed to alkenylic and carbinolic hydrogen atoms, respectively. The other hydrogen signals are registered between δ_H 2.0 and 0.8. The ^{13}C NMR spectrum showed signals at δ_C 79.1, characteristic of hydroxyl groups, and signals at δ_C 139.6 and 124.4, which are characteristic of the ursan skeleton. The ^{13}C NMR data of **5** are in agreement with the corresponding data described for α-amyrin (Costa et al., 2008).

The IR spectrum of the mixture of **5** and **6** showed strong absorptions at 3278, 1189, and 1096 cm^{-1}, which are characteristic of hydroxyl groups. The ^1H NMR spectrum showed signals at δ_H 5.17-5.10 and δ_H 4.67-4.56, which are attributed to alkenylic and carbinolic hydrogen atoms. The other hydrogen signals are registered between δ_H 2.0 and 0.8. The ^{13}C NMR spectrum showed signals at δ_C 139.6 and 124.4, which are characteristic of the ursan skeleton, and signals at δ_C 150.9 (non-hydrogenated carbon) and 109.3 (methylene carbon), which are characteristic of triterpenes with lupan skeleton. The ^{13}C NMR data of the mixture of **5** and **6** are in agreement with the corresponding data described for α-amyrin and lupeol (Costa et al., 2008), respectively.

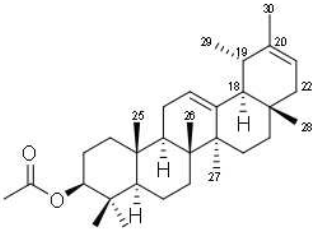
The IR spectrum of **7** showed strong absorptions at 1736, 1376, and 1217 cm^{-1}, which are characteristic of ester groups. The ^1H NMR spectrum showed a singlet signal at δ_H 5.46 and a doublet signal at δ_H 4.57 (J = 10.9 and 4.5 Hz), which are attributed to alkenylic and carbinolic hydrogen atoms. The other hydrogen signals are registered between δ_H 2.5 and 0.8. The ^{13}C NMR spectrum showed signals at δ_C 171.0, 145.5, and 116.3, which are characteristic of acetylated triterpenes with bauerenyl skeleton. The ^{13}C NMR data of **7** are in agreement with the corresponding data described for Δ^7-bauerenyl acetate (Mahato & Kundu, 1994).

The ^1H NMR spectrum of **8** only showed signals at δH 2.4 to 0.7, which are characteristic of aliphatic hydrogen atoms. The ^{13}C NMR spectrum showed a signal at δ_C 213.3, attributed to carbonylic carbon, and signals at δ_C 59.5 to 6.8. The ^{13}C NMR data of **8** are in agreement with the corresponding data described for friedelin (Mahato & Kundu, 1994).

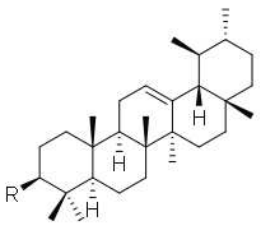
The ^1H NMR spectrum of the mixture of **9** and **10** showed multiplet signals at δ_H 5.36 and 5.03, both signals were attributed to alkenylic hydrogen atoms. The ^{13}C NMR spectrum showed signals at δ_C 140.8, 138.3, 129.3, and 121.7, which are in agreement with the corresponding data described for stigmasterol. The ^{13}C NMR data of **10** are similar to those of **9**, except for the signals attributed to the alkenylic carbon atoms, which were only recorded two signals at δ_C 140.8 and 121.7. These ^{13}C NMR data are in agreement with the corresponding data described for sitosterol (Costa et al., 2008).



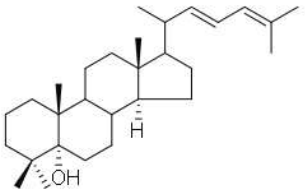
1 R = OAc
6 R = OH



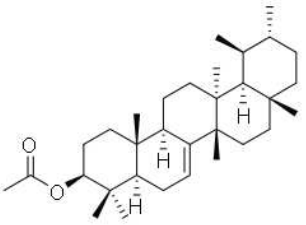
2



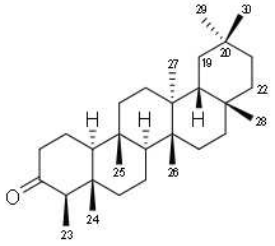
3 R = OAc
5 R = OH



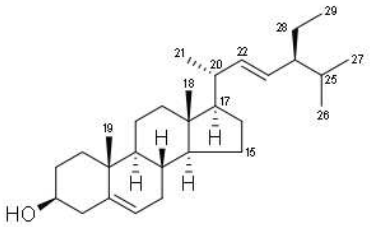
4



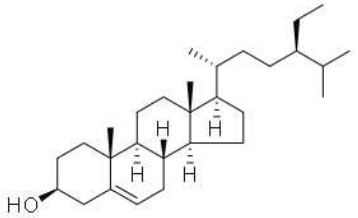
7



8



9



10

Mixture of 3-*O*-acetyl-lupeol (**1**) and 3-*O*-acetyl-pseudotaraxasterol (**2**): IR (cm^{-1}) *v* 2921, 2851, 1733, 1463, 1378, 1244, 1174, 1009, 979; ^1H NMR (400 MHz, $CDCl_3$) δ_H 5.36-5.12 (*m*), 4.69-4.45 (m); ^{13}C NMR data of **1** (100 MHz, $CDCl_3$) δ_C 174.0 (CO_2R), 151.2 (C-20), 109.6 (C-29), 81.3 (C-3), 55.6 (C-5), 50.6 (C-9), 48.3 (C-18), 47.9 (C-19), 43.1 (C-17), 42.4 (C-14), 41.2 (C-8), 40.3 (C-22), 38.8 (C-1/C-4), 38.0 (C-13), 37.1 (C-10), 35.2 (C-16), 34.5 (C-7), 29.8 (C-21), 28.2 (C-23), 27.7 (C-2), 27.3 (C-15), 25.3 (C-12), 21.2 (C-11), 19.5 (C-30), 18.3 (C-6), 18.0 (C-28), 16.2 (C-25), 16.0 (C-26), 15.8 (C-24), 14.5 (C-27); ^{13}C NMR data of **2** (100 MHz, $CDCl_3$) δ_C 171.0 (CO_2Me), 139.8 (C-20), 118.9 (C-21), 80.9 (C-3), 55.6 (C-5), 50.6 (C-9), 48.6 (C-18), 42.4 (C-14/C-22), 41.2 (C-8), 38.8 (C-1/C-4/C-13), 37.1 (C-10), 36.6 (C-16/C-19), 34.5 (C-7/C-17), 28.3 (C-23), 27.7 (C-2/C-12), 26.9 (C-15), 22.8 (C-29), 21.7 (C-30), 21.6 (C-11), 18.3 (C-6), 17.8 (C-28), 16.3 (C-25), 16.0 (C-26), 15.8 (C-24), 14.8 (C-27).

3-*O*-acetyl-α-amyrin (**3**): white solid; mp 238.0-240.0 °C; IR (cm^{-1}) *v* 2923, 1733, 1449, 1387, 1378, 1365, 1243, 1022, 1003, 984, 968; ^1H NMR (400 MHz, $CDCl_3$) δ_H 5.16 (*m*, H-12), 4.51 (*m*, H-3), 2.05 (*s*, CO_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 171.4 (CO_2Me), 139.6 (C-13), 124.3 (C-12), 81.0 (C-3), 59.0 (C-18), 55.2 (C-5), 47.7 (C-9), 42.1 (C-14), 41.5 (C-22), 40.0 (C-8), 39.6 (C-19/C-20), 38.7 (C-1/C-4), 36.9 (C-10), 33.7 (C-17), 32.9 (C-7), 31.2 (C-21), 28.7 (C-15), 28.1 (C-23/C-28), 27.2 (C-2), 26.6 (C-16), 23.3 (C-11/C-27), 21.3 (C-30), 18.3 (C-6), 17.4 (C-29), 16.9 (C-26), 15.6 (C-24/C-25); MS (70 eV) *m/z* (rel. int.) 468 [M^+] (2), 454 (1), 368 (1), 270 (2), 249 (2), 218 (100), 203 (26), 189 (22), 175 (6), 147 (7), 135 (14), 95 (14), 69 (11), 55 (9).

4,4-dimethyl-cholesta-22,24-dien-5-ol (**4**): MS (70 eV) *m/z* (rel. int.) 412 [M^+] (47), 395 (80), 136 (56), 122 (82), 93 (100), 55 (22), 43 (42).

α-amyrin (**5**): IR (cm^{-1}) *v* 3276, 3058, 2946, 2918, 2852, 1638, 1464, 1387, 1378, 1189, 1096, 1036, 992, 822; ^1H NMR (400 MHz, $CDCl_3$) δ_H 5.17 (m, H-12), 4.61 (m, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 139.6 (C-13), 124.4 (C-12), 79.1 (C-3), 59.0 (C-18), 55.2 (C-5), 47.7 (C-9), 42.1 (C-14), 41.5 (C-22), 40.0 (C-8), 39.6 (C-19/C-20), 38.7 (C-1/C-4), 36.9 (C-10), 33.7 (C-17), 32.9 (C-7), 31.2 (C-21), 28.7 (C-15), 28.1 (C-23/C-28), 27.2 (C-2), 26.6 (C-16), 23.3 (C-11/C-27), 21.4 (C-30), 18.3 (C-6), 17.4 (C-29), 16.9 (C-26), 15.6 (C-24/C-25); MS (70 eV) *m/z* (rel. int.) 426 [M^+] (12), 412 (2), 218 (100), 189 (35), 107 (70) , 81 (47).

Mixture of **5** and lupeol (**6**): IR (cm^{-1}) *v* 3278, 3078, 3058, 2946, 2918, 2852, 1646, 1464, 1387, 1378, 1189, 1096, 1036, 992, 880, 822; ^1H NMR (400 MHz, $CDCl_3$) δ_H 4.67 (*s*, H-29a), 4.56 (*s*, H-29b); ^{13}C NMR data of **6** (100 MHz, $CDCl_3$) δ_C 150.9 (C-20), 109.3 (C-29), 79.0 (C-3), 55.2 (C-5), 50.4 (C-9), 48.3 (C-18), 48.0 (C-19), 43.0 (C-17), 42.8 (C-14), 40.8 (C-8), 40.0 (C-22), 38.8 (C-4), 38.7 (C-1), 38.1 (C-13), 37.1 (C-10), 35.6 (C-16), 34.3 (C-7), 29.7 (C-21), 28.1 (C-23), 27.4 (C-2/C-15), 25.2 (C-12), 20.9 (C-11), 19.3 (C-30), 18.3 (C-6), 18.0 (C-28), 16.1 (C-25), 15.9 (C-26), 15.6 (C-24), 14.5 (C-27).

Δ^7-bauerenyl acetate (**7**): white solid; mp 267.2-275.4 °C; IR (cm^{-1}) *v* 2960, 2848, 1736, 1473, 1461, 1376, 1217, 736, 718; ^1H NMR (400 MHz, $CDCl_3$) δ_H 5.46 (m, H-7), 4.57 (*dd, J* = 10.9 and 4.5 Hz, H-3), 2.10 (s, CO_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 171.0 (CO_2Me), 145.5 (C-8), 116.3 (C-7), 81.2 (C-3), 54.9 (C-18), 50.6 (C-5), 48.1 (C-9), 41.3 (C-14), 38.0 (C-20), 37.8 (C-4/C-13), 37.7 (C-22), 36.6 (C-1), 35.4 (C-19), 35.1 (C-10), 32.4 (C-12), 32.1 (C-17/C-28), 31.6 (C-16), 29.2 (C-21), 28.9 (C-15), 27.5 (C-23), 25.7 (C-29), 24.2 (C-2), 24.0 (C-6), 23.7 (C-26), 22.7 (C-27), 22.6 (C-30), 16.9 (C-11), 15.8 (C-24), 13.1 (C-25).

friedelin (**8**): white solid; mp 257.8-259.0 °C; IR (cm^{-1}) *v* 2954, 2930, 2848, 1736, 1472, 1462, 1378, 1171, 730, 719; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 213.3 (C-3), 59.5 (C-10), 58.2 (C-4), 53.1 (C-8), 42.8 (C-18), 42.2 (C-5), 41.5 (C-2), 41.3 (C-6), 39.7 (C-13), 39.3 (C-22), 38.3 (C-14), 37.4 (C-9), 36.0 (C-16), 35.6 (C-11), 35.4 (C-19), 35.0 (C-29), 32.8 (C-21), 32.4 (C-15), 32.1 (C-28), 31.8 (C-30) 30.5 (C-12), 30.0 (C-17), 28.2 (C-20), 22.3 (C-1), 20.3 (C-26), 18.7 (C-27), 18.2 (C-7), 17.9 (C-25), 14.7 (C-24), 6.8 (C-23).

Mixture of stigmasterol (**9**) and sitosterol (**10**): ^1H NMR (400 MHz, $CDCl_3$) δ_H 5.36 (m, H-6), 5.03 (*m*, H-22/H-23), 3.53 (*m*, H-3); ^{13}C NMR data of **9** (100 MHz, $CDCl_3$) δ_C 140.8 (C-5), 138.3 (C-22), 129.3 (C-23), 121.7 (C-6), 71.8 (C-3), 56.9 (C-14), 56.0 (C-17), 51.2 (C-24), 50.1 (C-9), 42.3 (C-13), 42.2 (C-4), 40.5 (C-20), 39.7 (C-12), 37.2 (C-1), 36.5 (C-10), 31.9 (C-7/C-8/C-25), 31.7 (C-2), 29.0 (C-16), 25.4 (C-28), 24.4 (C-15), 21.2 (C-21/C-26), 21.1 (C-11), 19.4 (C-19), 19.0 (C-27), 12.1 (C-29), 12.0 (C-18); ^{13}C NMR data of **10** (100 MHz, $CDCl_3$) δ_C 140.8 (C-5), 121.7 (C-6), 71.6 (C-3), 56.9 (C-14), 56.0 (C-17), 50.1 (C-9), 45.2 (C-24), 42.3 (C-13), 42.2 (C-4), 39.7 (C-12), 37.2 (C-1), 36.5 (C-10), 35.5 (C-20), 33.4 (C-22), 31.9 (C-7/C-25), 31.7 (C-2), 31.4 (C-8), 29.0 (C-16), 25.5 (C-23), 22.6 (C-28), 24.4 (C-15), 21.2 (C-21), 20.9 (C-26), 21.1 (C-11), 19.4 (C-19), 18.6 (C-27), 12.1 (C-29), 12.0 (C-18).

Evaluation of the bactericidal and trypanocidal activities

The trypanocidal activities over the *T. cruzi* were 13±0.3% for **5** and 5±0.7% for LE, SE, and mixture of **5** and **6**. So, the extracts of LE and SE and the triterpene **5** and mixture of **5** and **6** showed low effect against intact bloodstream forms of the parasite (Oliveira et al., 2006).

In the agar diffusion tests, LE and SE were not active against *Bacillus cereus, Listeria monocytogenes, Escherichia coli, Salmonella typhimurium,* and *Citrobacter freundi.* Therefore, these extracts were active against *Staphylococcus aureus*, a very virulent bacterium, with great ability to produce localized infections that may become fatal if untreated (Shorr, 2007). Among the tested fractions, only **5** and mixture of **5** and **6** were active against *S. aureus* with a minimum inhibitory concentration greater than 1024 µg/mL. Although compound **3** only differs at C-3 position in relation to **5**, the former did not show bactericidal activity.

Conclusions

The present work describes the isolation of 3-*O*-acetyl-lupeol, 3-*O*-acetyl-α-amyrin, 4,4-dimethyl-cholesta-22,24-dien-5-ol, and Δ^7-bauerenyl acetate for the first time in the genus *Lychnophora*. The apolar extracts and triterpenes isolated from aerial parts of *L. pinaster* show low trypanocidal activity. As consequence, the trypanocidal activity previously described in the literature is not related to the triterpenes isolated from aerial parts of *Lychnophora pinaster.* Apolar extracts of leaves and stems and the triterpene α-amyrin, which was also isolated from these extracts, show effect against *S. aureus*. As acetyl α-amyrin did not show activity, the free hydroxyl group at C-3 may be related to the bactericidal activity of this triterpene.

Acknowledgements

The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico, Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, and Fundação de Amparo à Pesquisa do Estado de Minas Gerais for their financial support.

References

Alcântara AFC, Silveira D, Chiari E, Oliveira AB, Guimarães JE, Raslan DS 2005. Comparative analysis of the trypanocidal activity and chemical properties of *E*-lychnophoric acid and its derivatives using theoretical calculations. *Ecl Quim 30*: 37-45.

Al-Fatimi M, Wurster M, Schroder G, Lidequist U 2010. In vitro antimicrobial, cytotoxic, and radical scavenging activities and chemical constituents of the endemic *Thymus laevigatus* (Vahl). *Rec Nat Prod 4*: 49-63.

Borsato MLC, Grael CFF, Souza GEP, Lopes NP 2000. Analgesic activity of the lignans from *Lychnophora ericoides*. *Phytochemistry 55*: 809-813.

Costa HNR, Santos MC, Alcântara AFC, Silva MC, França RC, Piló-Veloso D 2008. Constituintes químicos e atividade antiedematogênica de *Peltodon radicans* (LAMIACEAE). *Quim Nova 31*: 744-750.

Cunha WR, Crevelin EJ, Arantes GM, Crotti AEM, Silva MLA, Furtado NAJC, Albuquerque S, Ferreira DS 2006. A study of the trypanocidal activity of triterpene acids isolated from *Miconia* species. *Phytother Res 20*: 474-478.

Ferraz Filha ZS, Vitolo IF, Fietto LG, Lombardi JA, Saúde-Guimarães DA 2006. Xanthine oxidase inhibitory activity of *Lychnophora* species from Brazil ("Arnica"). *J Ethnopharmacol 107*: 79-82.

Gao T, Yao H, Song H, Zhu Y, Liu C, Chen S 2010. Evaluating the feasibility of using candidate DNA barcodes in discrimining species of the large Asteraceae family. *BMC Evol Biol 10*: 324-330.

Grael CFF, Albuquerque S, Lopes JLC 2004. Chemical constituents of *Lychnophora pohlii* and trypanocidal activity of crude plant extracts and of isolated compounds. *Fitoterapia 76*: 73-82.

Guzzo LS, Saúde-Guimarães DA, Silva ACA, Lombardi JA, Guimarães HN, Grabe-Guimarães A 2008. Antinociceptive and anti-inflammatory activities of ethanolic extracts of *Lychnophora* species. *J Ethnopharmacol 116*: 120-124.

Lana EJL, Carazza F, Takahashi JA 2006. Antibacterial evaluation of 1,4-benzoquinone derivatives. *J Agric Food Chem 54*: 2053-2056.

Leite JPV, Oliveira AB, Lombardi JA, Filho JDS, Chiari E 2006. Trypanocidal activity of triterpenes from *Arrabidaea triplinervia* and derivatives. *Biol Pharm Bull 29*: 2307-2309.

Leite AC, Ambrozin ARP, Fernandes JB, Vieira PC, Silva MFGF, Albuquerque S 2008. Trypanocidal activity of limonoids and triterpenes from *Cedrela fissilis*. *Planta Med 74*: 1795-1799.

Mahato SB, Kundu AP 1994. ^{13}C NMR Spectra of pentacyclic triterpenoids - a compilation and some salient features. *Phytochemistry 37*: 1517-1575.

Mansanares ME, Forni-Martins ER, Semir J 2002. Chromosome numbers in the genus *Lychnophora* Mart. (Lychnophorinae, Vernonieae, Asteraceae). *Caryologia 55*: 367-374.

Merten J, Hennig A, Schwab P, Frohlich R, Tokalov SV, Gutzeit HO, Metz P 2006. Oxygenated 1,10-*seco*-eudesmanolides - enantioselective total synthesis of the antileukemic sesquiterpene lactones (–)-eriolanin

and (–)-eriolangin. *Eur J Org Chem 5*: 1144-1161.

Olea RSG, Roque NF 1990. Análise de misturas de triterpenos por RMN de ^{13}C. *Quim Nova 13*: 278-281.

Oliveira RB, Vaz ABM, Alves RO, Liarte DB, Donnici C, Romanha AJ, Zani CL 2006. Arylfurans as potential *Trypanosoma cruzi* trypanothione reductase inhibitors. *Mem Inst Oswaldo Cruz 101*: 169-173.

Robinson H 1999. Generic and sub-tribal classification of American vernonieae Smiths. *Contrib Bot 89*: 11-16.

Rosas LV, Cordeiro MSC, Campos FR, Nascimento SKR, Januario AH, Franca SC, Nomizo A, Toldo MPA, Albuquerque S, Pereira PS 2007. *In vitro* evaluation of the cytotoxic and trypanocidal activities of *Ampelozizyphus amazonicus* (Rhamnaceae). *Braz J Med Biol Res 40*: 663-670.

Santos MD, Gobbo-Neto L, Albarella L, Souza GEP, Lopes NP 2005. Analgesic activity of di-caffeoylquinic acids from roots of *Lychnophora ericoides* (Arnica da serra). *J Ethnopharmacol 96*: 545-549.

Saúde DA, Barrero AF, Oltra JE, Justicia J, Raslan DS, Silva EA 2002. Atividade antibacteriana de furanoeliangólidos. *Rev Bras Farmacogn 12*: 7-10.

Shorr AF 2007. Epidemiology and economic impact of meticillin-resistant *Staphylococcus aureus*. *Pharmacoeconomics 25*: 751-768.

Silveira D, Wagner H, Chiari E, Lombardi JA, Assunção AC, Oliveira AB, Raslan DS 2005. Biological activity of the aqueous extract of *Lychnophora pinaster* Mart. *Rev Bras Farmacogn 15*: 294-297.

Takahashi JA, Pereira CR, Pimenta LPS, Boaventura MAD, Silva LGFE 2006. Antibacterial activity of eight Brazilian Annonaceae plants. *Nat Prod Res 20*: 21-26.

Takeara R, Albuquerque S, Lopes NP, Lopes JLC 2003. Trypanocidal actitity of *Lychnophora staavioides* Mart. (Vernonieae, Asteraceae). *Phytomedicine 10*: 490-493.

Taleb-Contini SH, Santos WF, Mortari MR, Lopes NP, Lopes JLC 2008. Neuropharmacological effects in mice of *Lychnophora* species (Vernonieae, Asteraceae) and anticonvulsant activity of 4,5-di-*O*-[E]-caffeoylquinic acid isolated from the stem of *L. rupestris* and *L. staavioides*. *Basic Clin Pharmacol Taxicol 102*: 281-286.

Zhuang S, Hao C, Feng J, Zhang X 2010. Supercritical CO_2 extraction method and fungicidal activity of essential oil from *Mikania micrantha*. *Xibei Zhiwu Xuebao 30*: 163-169.

***Correspondence**

Antônio F. C. Alcântara
Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais Pampulha, 31260-901 Belo Horizonte-MG, Brazil
aalcantara@zeus.qui.ufmg.br
Tel.: +55 31 3409 5728
Fax: +55 31 3409 5700