academicJournals

Vol. 8(8), pp. 353-360, 25 February, 2014
DOI: 10.5897/JMPR2013.5153
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Phytochemical investigation and antioxidant activity of extracts of *Lecythis pisonis* Camb.

Éverton Leandro de França Ferreira[1], Thamires Silva Mascarenhas[1], Jocélia Pereira de Carvalho Oliveira[1], Mariana Helena Chaves[1]*, Bruno Quirino Araújo[2] and Alberto José Cavalheiro[3]

[1]Universidade Federal do Piauí, Departamento de Química, Teresina – PI, Brazil.
[2]Instituto de Química, Universidade Estadual de Campinas, Campinas – SP, Brazil.
[3]Departamento de Química Orgânica, Instituto de Química, Universidade Estadual Paulista Júlio de Mesquita Filho, Araraquara-SP, Brazil.

Received 4 July, 2013; Accepted 9 February, 2014

Phytochemical investigation of the ethanol extract of leaves, twigs and fruit shell of *Lecythis pisonis* Camb. revealed the presence of squalene, α- e β-amyrin, lupeol, 3β-friedelinol, ursolic and oleanolic acids, (*E*)-phytol, sitosterol, stigmasterol, campesterol, quercetin-3-*O*-rutinoside and kaempferol-3-*O*-rustinoside. Structural elucidation was achieved using ultraviolet (UV), nuclear magnetic resonance (NMR), and mass spectrometry. This is the first report of the occurrence of flavonoids, together with squalene, lupeol and campesterol in *L. pisonis*. The EtOH extract of the leaves showed high antioxidant activity, which can be associated in part with the high level of phenols and flavonoids.

Key words: Lecythidaceae, *Lecythis pisonis*, triterpenes, flavonoids, antioxidant activity, total phenolic content, total flavonoid content.

INTRODUCTION

Plants of the Lecythidaceae family are representatives of many neotropical forests in the Americas, Africa and Asia. This family has about 25 genera and 400 species in three subfamilies: Foetidioideae, Planchonioideae and Lecythidoideae (Mori, 2001). The genus *Lecythis* is little studied from the chemical point of view. There are reports in the literature on the chemical speciation of selenium (Se) in nuts of *Lecythis minor* (Dernovics et al., 2007), chemical analysis of the essential oils from flowers of *Lecythis usitata* (Andrade et al., 2000) and leaves of *Lecythis persistens* and *Lecythis poiteau* (Courtois et al., 2009). The species *Lecythis pisonis* Camb popularly known as sapucaia, is distributed in Brazil, within the state of Piaui and from Pernambuco to São Paulo as well as in the Amazon region (Corrêa, 1978). Traditionally, the infusion prepared from the bark of the tree is astringent and used in the treatment of diarrhea, while the leaves are used as diuretic and tonicardiac in tea or infusion or in baths to relieve itching (pruritus) and the fruits are used in the treatment of diarrhea and syphilis (Braga et al.

*Corresponding author. E-mail: mariana@ufpi.edu.br. Tel: 5586 3237 1584.

2007; Denadai, 2006). The phytochemical study of the leaves of this species led to the isolation of pentacyclic triterpenoids, phytol, sitosterol and stigmasterol (Oliveira et al., 2012). The ethanol extract and the mixture of ursolic and oleanolic acids obtained from the leaves demonstrated antipruritic and cytotoxic activities (Silva et al., 2012; Oliveira et al., 2012). Other studies also showed that ethanol extract, eterea fraction and mixture of ursolic and oleanolic acids from leaves of *L. pisonis* exhibited antinociceptive activity in models of acute pain in mice (Brandão et al., 2013).

As part of our studies aimed at exploring the chemical constituents and pharmacological potential of plant species of the cerrado and transition area in the state of Piauí, this present work describes the isolation and identification of chemical constituents from the ethanol extract of leaves, twigs and fruit shell of *L. pisonis* as well as the evaluation of the antioxidant potential and determination of content phenols and flavonoids total.

MATERIALS AND METHODS

General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA and Bruker Avance III spectrometer at 500/400 and 125/100 MHz, respectively, using $CDCl_3$ or DMSO-d_6 as solvents. The samples were analysed by gas chromatography–mass spectrometry (GC-MS) on an Agilent Technologies 7890A GC system coupled to a 5975 VLMSD mass spectrometer equipped with a 7683B series injector device and DB-5 column (J&W, 30 m × 250 mm × 0.25 mm), with injector temperature at 250°C and temperature at the interface of 310°C. The injected volume was 1.0 ml (5 mg ml^{-1}) in the split mode (10:1). Helium was used as carrier gas, at a velocity of 1 ml min^{-1}. Column temperature was initially maintained at 200°C for 4 min, followed by a heating gradient of 6°C min^{-1} until reaching 290°C. This temperature was maintained for 15 min after which a new heating gradient of 2°C min^{-1} was applied until reaching 305°C. This temperature was maintained for 5 min. The obtained mass spectra were compared to library data Nist 0.5. The absorption measurements were determined by an UV-Vis spectrophotometer PerkinElmer, Lambda 25. The chromatographic plates were prepared using Fluka silica gel G and the revealed were made by spraying the plates with a solution of $Ce(SO_4)_2$. The atmospheric pressure chromatographic columns were prepared with silica gel 60 (70 to 230 mesh) from Acros Organics or Sephadex LH-20 (Aldrich). For low-pressure chromatography column Büchi Switzerland with silica gel (40-60 µm; 12 × 150 mm) coupled to a Büchi B-688 pump was used. The semipreparative high performance liquid chromatography (HPLC) was performed on a Shimadizu LC-6AD system equipped with a Phenomenex column (Luna C18, 5 µm, 150 × 21.2 mm).

Plant

The leaves and twigs *L. pisonis* Camb. were collected in July, 2008 and fruit shell in July, 2010, on the Campus Ministro Petronio Portela of the Universidade Federal do Piauí - UFPI, in the city of Teresina, Piauí State, (South latitude = 05° 02' 53.2", West longitude = 42° 47' 16.8", at the level of 68 m). A voucher specimen was identified and deposited in the Herbarium Graziela Barroso at the UFPI, under accession number TEPB 26488.

Preparation of extracts and partition

The plant material consisting of leaves (2.0 kg), twigs (1.2 kg) and fruit shell (3.0 kg) of *L. pisonis* was air-dried, crushed and extracted for six consecutive times with ethanol at room temperature. The ethanol was removed on a rotary evaporator and the residual water by lyophilization, giving 272 g (13.5%), 72 g (5.9%) and 26 g (0.9%) of EtOH extracts, respectively. The EtOH extracts of leaves (200 g) and fruit shell (24 g) were suspended in H_2O:MeOH (3:2) and subjected to partition with *n*-hexane, ethyl ether and EtOAc, successively, resulting in the following fractions: hexane (60 g, 30% and 5.9 g, 24.5%), ether (24 g, 12% and 2.8 g, 11.6%), EtOAc (21 g, 10.5% and 1.8 g, 7.7%), aqueous (70 g, 37.5% and 10.8 g, 44.9%) and a precipitate separated from the EtOAc phase of the leaves extract (ppt-EtOAc; 10 g, 5%). The EtOH extract of the twigs (60 g) was suspended in H_2O:MeOH (3:2) and partitioned with hexane and EtOAc, giving the following fractions: hexane (5.3 g, 8.8%), EtOAc (16.3 g, 27.1%) and aqueous (30 g, 50%).

Isolation and fractionation of the constituents

The hexane fractions of leaves (8.0 g), twigs (4.1 g) and fruit shell (5.2 g) were submitted to column chromatography (5.0 × 50 cm; 3.0 × 50 cm and 3.0 × 50 cm) of silica gel, eluted with *n*-hexane:EtOAc: (100:0), (98:2), (95:5), (9:1), (8:2) and (7:3) providing 102, 100 and 60 fractions, respectively. After removal of the solvent on a rotary evaporator, the fractions were regrouped according to thin layered chromatography (TLC) analysis. Fractionation of the *n*-hexane fraction of leaves provided 11 groups (A_1 to K_1). Group B_1 (fractions 6 to 7, 89 mg) was purified on Sephadex LH-20 eluted with *n*-hexane:CH_2Cl_2 (1:4) giving 32 mg of compound 1. Group D_1 (fraction 25, 175 mg) was suspended in *n*-hexane providing, after separation of the supernatant, an amorphous solid corresponding to the compound 2 (112 mg). Groups F_1 (fractions 34 to 38, 839 mg) and H_1 (fractions 43 to 59, 714 mg) were purified on Sephadex LH-20 eluted with *n*-hexane:CH_2Cl_2 (1:4) providing 124 mg of the mixture of compounds 3 to 5 and 11 and 31 mg of a mixture of compounds 8 and 9, respectively. Group J_1 (fractions 98 to 100, 350 mg) was purified on Sephadex LH-20 column eluted with *n*-hexane:CH_2Cl_2 (1:4) and CH_2Cl_2-acetone (3:2), giving 42 mg of a mixture of compounds 6 and 7.

For GC-MS analysis, an aliquot of 5 mg of these substances was treated with a solution of diazomethane in ethyl ether providing the methylated derivatives. Fractionation of the hexanic fraction of the twigs provided 7 groups (A_2 to G_2). Groups B_2 (fraction 30, 107 mg) and D_2 (fractions 46 to 54, 138 mg) were purified on Sephadex LH-20 column eluted with n-hexane:CH_2Cl_2 (1:4) giving 32 mg of the mixture of compounds 3 to 5 and 11 and 25 mg of the mixture of compounds 8 to 10, respectively. Group F_2 (fractions 96 to 97, 48.6 mg) was purified on Sephadex LH-20 column eluted with *n*-hexane:CH_2Cl_2 (1:4) and CH_2Cl_2:acetone (3:2), giving 14.5 mg of the mixture of compound 6 and 7. Fractionation of the hexanic fraction of fruit shell provided 5 groups (A_3 to E_3). Groups B_3 (fractions 37 to 38, 267 mg) and D_3 (fractions 46 to 51, 300 mg) were purified on Sephadex LH-20 eluted with n-hexane: CH_2Cl_2 (1:4) giving 48 mg of a mixture of compounds 3 to 5 and 85 mg of the mixture of 8 and 9, respectively.

The EtOAc fraction leaves (10 g) was submitted to column chromatography (5.0 × 50 cm) of silica gel, eluted with $CHCl_3$:

MeOH: (100:0), (95:5), (9:1), (8:2) and (1:1), giving 60 fractions.

After removal of solvent on a rotary evaporator and analysis by TLC, the fractions were pooled into 5 groups (A to E). Group B (fractions 8 to 15, 375 mg) was fractionated on columns of Sephadex LH-20, eluted with CH_2Cl_2:acetone (3:2) and silica gel low pressure eluted with *n*-hexane:$CHCl_3$:MeOH (50:46.5:3.5) giving 15 mg of a mixture of compounds 6 and 7. Group D (fractions 42 to 47, 768 mg) was applied on a column of Sephadex LH-20 eluted with MeOH giving 40 fractions which were pooled into 5 groups (D_1 to D_5). Group D_2 (fractions 20 to 25, 417 mg) was purified by semi-preparative HPLC eluted with MeOH:H_2O (42:58) to provide compounds 12 (85 mg) and 13 (9.5 mg).

Squalene (1): ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.8 (C-1), 131.4 (C-2), 124.5 (C-3), 26.9 (C-4), 39.9 (C-5), 135.3 (C-6), 124.5 (C-7), 26.8 (C-8), 39.9 (C-9), 135.1 (C-10), 124.5 (C-11), 28.4 (C-12), 28.4 (C-13), 124.5 (C-14), 135.1 (C-15), 39.9 (C-16), 26.8 (C-17), 124.5 (C-18), 135.3 (C-19), 39.9 (C-20), 26.9 (C-21), 124.6 (C-22), 131.4 (C-23), 25.8 (C-24), 17.8 (C-25), 16.1 (C-26), 16.1 (C-27), 16.1 (C-28), 16.1 (C-29), 17.8 (C-30).

3β-Friedelinol (2): ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.7 (C-1), 36.9 (C-2), 72.4 (C-3), 49.1 (C-4), 38.3 (C-5), 41.6 (C-6), 17.5 (C-7), 53.1 (C-8), 37.7 (C-9), 61.2 (C-10), 35.5 (C-11), 30.5 (C-12), 38.7 (C-13), 39.6 (C-14), 32.2 (C-15), 35.9 (C-16), 29.9 (C-17), 42.7 (C-18), 35.2 (C-19), 28.1 (C-20), 32.7 (C-21), 39.2 (C-22), 11.4 (C-23), 16.1 (C-24), 18.1 (C-25), 19.9 (C-26), 18.5 (C-27), 31.9 (C-28), 34.8 (C-29), 31.6 (C-30).

Lupeol (3): EIMS: *m/z* (rel. int.) 426 (9, [$M^{+\bullet}$]), 411 (3), 207 (54), 189 (100), 175 (24), 161 (17), 135 (59), 121 (80), 95 (82), 55 (52).

α-Amyrin (4): ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.7 (C-1), 27.1 (C-2), 79.2 (C-3), 38.7 (C-4), 55.3 (C-5), 18.5 (C-6), 32.9 (C-7), 40.0 (C-8), 47.8 (C-9), 36.8 (C-10), 23.4 (C-11), 124.5 (C-12), 139.7 (C-13), 42.2 (C-14), 28.9 (C-15), 26.7 (C-16), 33.9 (C-17), 59.2 (C-18), 39.5 (C-19), 39.5 (C-20), 31.2 (C-21), 41.6 (C-22), 28.1 (C-23), 15.6 (C-24), 15.6 (C-25), 16.9 (C-26), 23.4 (C-27), 28.1 (C-28), 17.6 (C-29), 21.5 (C-30).

β-Amyrin (5): ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.7 (C-1), 27.4 (C-2), 79.2 (C-3), 38.9 (C-4), 55.3 (C-5), 18.5 (C-6), 32.8 (C-7), 38.9 (C-8), 47.8 (C-9), 37.6 (C-10), 23.7 (C-11), 121.8 (C-12), 145.3 (C-13), 41.8 (C-14), 26.1 (C-15), 27.1 (C-16), 32.6 (C-17), 47.3 (C-18), 46.9 (C-19), 31.2 (C-20), 34.8 (C-21), 37.3 (C-22), 28.2 (C-23), 15.6 (C-24), 15.6 (C-25), 16.9 (C-26), 26.1 (C-27), 28.5 (C-28), 33.5 (C-29), 23.8 (C-30).

Ursolic acid (6): ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.7 (C-1), 23.0 (C-2), 78.8 (C-3), 36.9 (C-4), 55.2 (C-5), 18.3 (C-6), 33.9 (C-7), 39.4 (C-8), 47.5 (C-9), 36.9 (C-10), 23.3 (C-11), 125.5 (C-12), 138.1 (C-13), 42.0 (C-14), 27.7 (C-15), 23.0 (C-16), 47.8 (C-17), 52.8 (C-18), 39.1 (C-19), 41.2 (C-20), 30.7 (C-21), 36.8 (C-22), 27.9 (C-23), 16.8 (C-24), 15.5 (C-25), 16.8 (C-26), 23.5 (C-27), 181.0 (C-28), 16.9 (C-29), 21.1 (C-30).

Oleanolic acid (7): ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.5 (C-1), 27.7 (C-2), 78.8 (C-3), 38.6 (C-4), 55.2 (C-5), 18.3 (C-6), 32.5 (C-7), 39.4 (C-8), 47.5 (C-9), 36.9 (C-10), 23.0 (C-11), 122.3 (C-12), 143.5 (C-13), 41.7 (C-14), 27.7 (C-15), 23.3 (C-16), 46.4 (C-17), 41.2 (C-18), 45.9 (C-19), 30.6 (C-20), 33.9 (C-21), 32.5 (C-22), 27.9 (C-23), 15.5 (C-24), 15.4 (C-25), 16.9 (C-26), 25.8 (C-27), 181.2 (C-28), 33.0 (C-29), 23.5 (C-30).

Sitosterol (8): ^{13}C NMR (100 MHz, $CDCl_3$): δ 37.4 (C-1), 31.8 (C-2), 71.9 (C-3), 42.4 (C-4), 140.9 (C-5), 121.9 (C-6), 32.0 (C-7), 32.0 (C-8), 50.3 (C-9), 36.6 (C-10), 21.2 (C-11), 39.9 (C-12), 42.5 (C-13), 56.9 (C-14), 24.4 (C-15), 28.4 (C-16), 56.2 (C-17), 12.0 (C-18), 19.5 (C-19), 36.3 (C-20), 18.9 (C-21), 34.0 (C-22), 26.2 (C-23), 45.9 (C-24), 29.3 (C-25), 19.9 (C-26), 19.2 (C-27), 22.8 (C-28), 12.0 (C-29).

Stigmasterol (9): ^{13}C NMR (100 MHz, $CDCl_3$): δ 37.4 (C-1), 31.8 (C-2), 71.9 (C-3), 42.4 (C-4), 140.9 (C-5), 121.9 (C-6), 32.0 (C-7), 32.0 (C-8), 50.3 (C-9), 36.6 (C-10), 21.2 (C-11), 39.9 (C-12), 42.5 (C-13), 56.9 (C-14), 24.4 (C-15), 28.4 (C-16), 56.2 (C-17), 12.0 (C-18), 19.5 (C-19), 36.9 (C-20), 21.2 (C-21), 138.5 (C-22), 129.4 (C-23), 50.3 (C-24), 31.8 (C-25), 21.2 (C-26), 18.9 (C-27), 25.5 (C-28), 12.2 (C-29).

Campesterol (10): EIMS: *m/z* (rel. int.) 400 (25, [$M^{+\bullet}$]), 382 (70), 315 (25), 289 (15), 273 (13), 255 (38), 55 (100).

(*E*)-phytol (11): ^{13}C NMR (100 MHz, $CDCl_3$): δ 59.6 (C-1), 123.2 (C-2), 140.5 (C-3), 39.5 (C-4), 25.3 (C-5), 36.8 (C-6), 32.7 (C-7), 37.4 (C-8), 24.6 (C-9), 37.4 (C-10), 32.9 (C-11), 37.4 (C-12), 24.9 (C-13), 39.5 (C-14), 28.1 (C-15), 22.8 (C-16), 22.8 (C-17), 19.9 (C-18), 19.9 (C-19), 22.8 (C-20).

Quercetin-3-O-rutinoside (12): ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.6 (d, J 2.0 Hz, H-2'), 6.19 (d, *J* 2.0 Hz, H-6), 6.38 (d, *J* 2.0 Hz, H-8), 7.55 (d, J 2.0 Hz, H-2'), 6.84 (d, J 8.5 Hz, H-5'), 7.65 (dd; *J* 2.5 and 8.5 Hz, H-6'), 5.34 (d, *J* 7.5 Hz, H-1''), 4.39 (d, *J* 1.5 Hz, H-1'''), 1.18 (d, *J* 6.0 Hz, H-6'''), 2.75-3.70 (H-2'' to H-6'', H-2''' to H-5'''). ^{13}C NMR (125 MHz, DMSO-d_6): δ 156.4 (C-2), 133.1 (C-3), 177.4 (C-4), 161.2 (C-5), 98.7 (C-6), 164.3 (C-7), 93.7 (C-8), 156.4 (C-9), 104.0 (C-10), 121.6 (C-1'), 116.3 (C-2'), 144.7 (C-3'), 148.5 (C-4'), 115.2 (C-5'), 121.9 (C-6'), 101.4 (C-1''), 74.2 (C-2''), 76.5 (C-3''), 70.4 (C-4''), 76.0 (C-5''), 68.3 (C-6''), 100.8 (C-1'''), 70.6 (C-2'''), 70.6 (C-3'''), 71.9 (C-4'''), 68.3 (C-5'''), 17.9 (C-6''').

Kaempferol-3-O-rutenosideo (13): ^{1}H NMR (500 MHz, DMSO-d_6), δ 12.8 (s, 5-OH), 6.18 (d, *J* 2.0 Hz, H-6), 6.41 (d, *J* 2.0 Hz, H-8), 7.98 (d, *J* 9.0 Hz, H-2'/H-6'), 6.88 (d, *J* 9.0 Hz; H-3'/H-5'), 5.30 (d, *J* 7.5 Hz, H-1''), 4.38 (d, *J* 1.0 Hz, H-1'''), 0.98 (d, *J* 6.0 Hz, H-6'''), 3.00-3.90 (H-2'' to H-6'', H-2''' to H-5'''). ^{13}C NMR (125 MHz, DMSO-d_6), δ 157.2 (C-2), 133.3 (C-3), 177.9 (C-4), 161.5 (C-5), 99.3 (C-6), 164.7 (C-7), 94.1 (C-8), 156.7 (C-9), 104.1 (C-10), 121.3 (C-1'), 131.4 (C-2'/6'), 115.7 (C-3'/5'), 160.3 (C-4'), 101.6 (C-1''), 74.5 (C-2''), 76.8 (C-3''), 70.7 (C-4''), 76.2 (C-5''), 68.5 (C-6''), 100.9 (C-1'''), 70.8 (C-2'''), 70.9 (C-3'''), 71.9 (C-4'''), 68.5 (C-5'''), 18.0 (C-6''').

Determination of antioxidant activity and dosage of total phenolics and flavonoids

The antioxidant activity was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical consumption, and the quantification of phenolic compounds determined by Folin-Ciocalteu method and expressed in gallic acid equivalent (GAE) according to Sousa et al. (2007) and Costa et al. (2010). The total flavonoid content was determined by molecular absorption spectrometry, following the methodology described by Sobrinho et al. (2010). Stock solutions of EtOH extracts and fractions (1000 µg ml^{-1}) were prepared and then an aliquot of 300 µl of these solutions was transferred to 10 ml flasks to which 0.24 ml of acetic acid, 4 ml of a methanolic solution of pyridine at 20% and 1 ml of a methanolic solution of aluminum chloride (50 mg ml^{-1}) were added, completing the volume with distilled water. Control was prepared in parallel.

Table 1. Chemical constituents of hexane fractions of *Lecythis pisonis.*

Compounds	Hexane fractions		
	Leaves	Twigs	Fruit shell
Squalene (1)	X	-	-
3β-Friedelinol (2)	X	-	-
Lupeol (3)	X[a]	X	X
α-Amyrin (4)	X	X	X
β-Amyrin (5)	X	X	X
Ursolic acid (6)	X	X	-
Oleanolic acid (7)	X	X	-
Sitosterol (8)	X	X	X
Stigmasterol (9)	X	X	X
Campesterol (10)	-	X[a]	-
(*E*)-phytol (11)	X	X	-

[a]Only identified by GC-MS.

Table 2. Antioxidant activity (EC_{50}), total phenolics (TP) and flavonoids content (TFC) of *Lecythis pisonis* extracts.

Samples	EC_{50} (μg ml^{-1}±SD)	TP (mg GAE g^{-1} DPM±SD)	TFC (mg RE g^{-1} DPM±SD)
EtOH leaves	49.04±1.65	56.78±0.13	30.04±0.02
EtOH twigs	71.52±3.11	29.52±0.30	3.77±0.19
EtOH fruit shell	ND	0.88±0.01	0.73±0.00
Rutin	47.08±4.65	-	-

EC_{50} = effective concentration; GAE = gallic acid equivalent; RE = rutin equivalent; DPM = dried plant material; SD = standard deviation; ND = not determined.

After 30 min, the absorbance of samples was measured at 420 nm using glass cuvets. The total flavonoid content (TFC) was determined by interpolating the absorbance of the samples against a calibration curve constructed with standard rutin at concentrations of 3, 6.5, 10, 13.5, 17 and 21 mg l^{-1}, obtained from a stock solution of 1000 mg l^{-1} in MeOH:H_2O (7:3). To each flask containing 10 ml of these solutions, 0.24 ml of acetic acid, 4 ml of a methanolic solution of pyridine at 20% and 1 ml of methanol solution of aluminum chloride (50 mg ml^{-1}) were added completing with distilled water. After 30 min at room temperature, lecture was performed using a spectrophotometer at 420 nm. Values are expressed as milligrams of equivalent rutin per gram of dried plant material (mg of ER g^{-1} of DPM). The straight line equation is: $A = 0.0262C - 0.0072$, where A is the absorbance, C is concentration and linear correlation coefficient of $r^2 = 0.999$. All analyzes were performed in triplicate (n = 3).

RESULTS

The phytochemical study of hexane fractions obtained after partition of the EtOH extract of the leaves, twigs and fruit shell of *L. pisonis* resulted in the isolation and identification by GC-MS, ^1H and ^{13}C NMR of seven triterpenoids (1 to 7), three steroids (8 to 10) and a diterpenoid (11) listed in Table 1 and Figure 1. The triterpenoid lupeol (3), α- and β-amyrin (4 and 5) and steroids sitosterol (8) and stigmasterol (9) were identified in all samples. Squalene (1) and 3β-friedelinol (2) were identified only in leaves, while campesterol (10) was identified only in the twigs. The ursolic (6) and oleanolic (7) acids were identified in the leaves and twigs, but were not obtained from fruit shells. Fractionation of the EtOAc fraction, resulting from the partition of EtOH extract of the leaves of *L. pisonis* resulted in the isolation of the flavonoids quercetin-3-*O*-rutinoside (12) and kaempferol-3-*O*-rutinoside (13) (Figure 1). The structural identification of these compounds was based on analysis of UV spectra, NMR (1D and 2D).

The antioxidant activity (AA%) of the EtOH extracts of leaves, twigs and fruit shells of *L. pisonis* and positive control (rutin) in concentrations ranging from 25 to 250 μg ml^{-1}, are shown in Figure 2. The extracts and control showed concentration dependent antioxidant activity. Table 2 presents the results of the evaluation of EtOH extracts of leaves, twigs and fruit shells on the content of flavonoids and phenolics and total antioxidant activity expressed by EC_{50}. The EtOH extract of the fruit shell

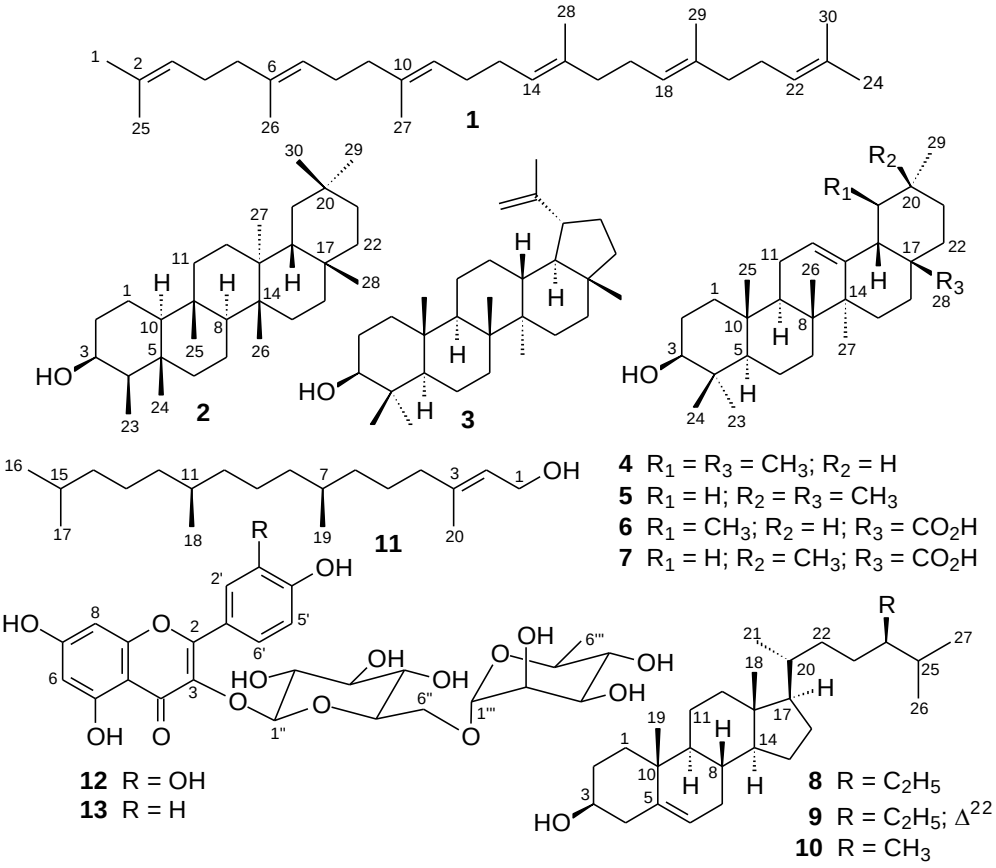

Figure 1. Substances isolated from extracts *Lecythis pisonis.*

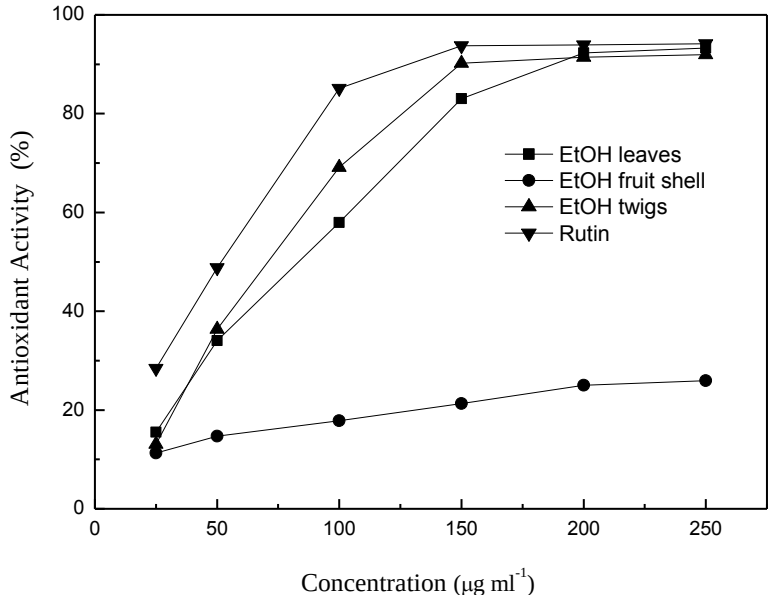

Figure 2. Percentage of antioxidant activity (AA%) of EtOH extracts of leaves, twigs and fruit shell of *L. pisonis*. Positive control: rutin.

Table 3. Total phenols (TP) and total flavonoid content (TFC) in fractions of a partition of ethanol extracts of *L. pisonis.*

Fraction	TP (mg GAE g^{-1} DPM±SD)	TFC (mg RE g^{-1} DPM±SD)
Hexane leaves	ND	ND
Ether leaves	5.53±0.12	4.39±0.00
EtOAc leaves	6.40±0.04	5.21±0.00
Aqueous leaves	42.81±1.02	10.59±0.01
Hexane twigs	0.35±0.00	ND
EtOAc twigs	7.56±0.13	2.37±0.40
Aqueous twigs	10.81±0.22	6.51±0.22

GAE = gallic acid equivalent; RE = rutin equivalent; DPM = dried plant material; SD = standard deviation; ND = not determined.

showed no significant antioxidant activity. The total phenols and flavonoid contents, determined in relation to the dry plant material, lying in the range 0.88 to 56.78 mg of GAE g^{-1} and of 0.73 to 30.04 mg of ER g^{-1}, respectively, with the highest concentrations of these constituents being obtained from the EtOH extract of the leaves (Table 2). Table 3 shows the levels of total phenols and flavonoids of partition fractions of EtOH extracts of leaves and twigs, which proved to be richer in these constituents. Significant differences between fractions were observed ($p < 0.05$). The highest total phenols and flavonoids contents were recorded for the aqueous fraction of the leaves; however it was not possible to determine the flavonoid in hexane fractions of leaves and twigs due to the absence or low content of these constituents.

DISCUSSION

The structural identification of substances 1 to 11 was performed by GC-MS analysis, ^{1}H and ^{13}C NMR and comparison with literature data (Olea and Roque, 1990; Rahman and Ahmad, 1992; Mahato and Kundu, 1994; Junges et al., 2000; Salazar et al., 2000; De-Ekankul et al., 2003). The lupeol (3, leaves) and campesterol (10, twigs) were identified solely on analysis by GC-MS due to the low concentration of these substances in samples. Squalene, lupeol and campesterol are reported for the first time in *L. pisonis*.

The UV spectra of compounds 12 and 13 showed two absorptions maxima at 255/265 and 355/346 nm characteristic of flavonol. ^{1}H NMR spectra (DMSO-d_6) showed typical signals of glycosylated flavonols at δ 5.34/5.30 (d, *J* = 7.5 Hz, H-1'') related to β-D-glucose, δ 4.39/4.38 (d, *J* = 1.5/1.0 Hz, H-1''') assigned to α-L-rhamnose, signals at δ 12.6/12.8 regarding the hydroxyl hydrogens at C-5 chelated to carbonyl and two doublets at δ 6.19/6.18 (*J* = 2.0 Hz, H-6) and δ 6.38/6.41 (*J* = 2.0 Hz, H-8), for the aromatic ring A. The main difference observed in the ^{1}H NMR spectra of these compounds concerned the B ring, as flavonoid 12 showed signals corresponding to three hydrogens, a double doublet at δ 7.65 (1H; *J* = 2.5 and 8.5 Hz, H-6') and two doublets at δ 7.55 (1H; *J* = 2.0 Hz, H-2') and δ 6.84 (1H; *J* = 8.5 Hz, H-5'), characteristic of the 3',4'-dihydroxyflavonol (Fathiazad et al. 2006). However, flavonoid 13 showed signals for four hydrogen at δ 7.98 (2H; d, *J* = 9.0 Hz, H-2'/H-6') and 6.88 (2H; d, *J* = 9.0 Hz, H-3'/H-5') characteristic of 4'-hydroxyflavonol (Song et al., 2007). The interglycosidic connection [rhamnopyranosyl(α1'''→6'')glucopyranose] was defined in the heteronuclear multiple bond correlation (HMBC) contour map by the correlation of anomeric hydrogen signal at δ 4.39/4.38 (H-1''') with δ 68.3/68.8 (C-6''), while the location of the diglucoside rutinose moiety [rhamnopyranosyl(α1'''→6'')glucopyranose] at C-3 of the aglycone was determined by the correlation of the signal of the anomeric hydrogen at δ 5.30/5.34 (H-1'') with δ 133.1/133.3 (C-3). A comparison of the NMR data obtained with those reported in literature (Fathiazad et al., 2006; Song et al., 2007) allowed the identification of compounds 12 and 13 as quercetin-3-*O*-rutinoside (rutin) and kaempferol-3-*O*-rutinoside (nicotiflorin), respectively. This is the first report of the identification of flavonoids 12 and 13 in *L. pisonis* and it is also the first described occurrence of the flavonoid kaempferol-3-*O*-rutinoside in Lecythidaceae.

The EtOH extracts of leaves and twigs, in concentrations of 200 and 250 µg ml^{-1} showed antioxidant activity comparable to percentage of the positive control (rutin), while the EtOH extract of the fruit shell was the least active in all concentrations tested. Antioxidant activity was also evaluated by EC_{50}, thus the EtOH extract of the leaves (EC_{50} = 49.04 ± 1.65 µg ml^{-1}) was comparable to the positive control rutin (EC_{50} = 47.08 ± 4.65 µg ml^{-1}).

According to Rufino et al. (2010), the content of phenolic compounds, expressed as gallic acid equivalents

per gram of dried plant material may be classified into low (< 10 mg GAE g^{-1}), medium (10 to 50 mg GAE g^{-1}) and high (> 50 mg GAE g^{-1}). In this study, the content of phenolic compounds was high for the EtOH extract of leaves, medium for the twigs and low for the fruit shell. A positive correlation between the EC_{50} and the content of phenols and flavonoids of EtOH extracts of leaves, twigs and fruit shells was observed. This behavior is generally expected, considering that the phenolic compounds and in particular flavonoids are free radicals scavengers and consequently exhibit antioxidant properties.

The phenolic contents for the fractional partition EtOH extract of the leaves ranged from 0.06 to 42.81 mg GAE g^{-1} of DPM while flavonoids contents ranged from 2.37 to 10.59 mg ER g^{-1} of DPM. Although the total phenols of EtOAc fraction of leaves was lower than in the EtOAc fraction of the twigs, the content of flavonoids was higher, being explained partly by the presence of flavonoids quercetin-3-*O*-rutinoside and kaempferol-3-*O*-rutinoside, that were detected in the EtOAc fraction of leaves but not from twigs, according to TLC analysis.

Conclusions

The phytochemical study of the hexane fractions of the leaves, twigs and fruit shells of *L. pisonis* resulted in the isolation and identification of seven triterpenoids: squalene (1), 3β-friedelinol (2), lupeol (3), α- and β-amyrin (4 and 5) ursolic and oleanolic acids (6 and 7), three steroids: sitosterol (8), stigmasterol (9) and campesterol (10) and a diterpenoid, the (*E*)-phytol (11). The EtOH extract of the leaves showed the highest antioxidant activity and the highest levels of total phenolics and flavonoids. These results can be partly explained by the presence of flavonoids, quercetin-3-*O*-rutinoside (12) and kaempferol-3-*O*-rutinoside (13), isolated from the EtOAc fraction resulting from the partition of the EtOH extract of leaves. The two flavonoids as well as lupeol, squalene and campesterol are being reported for the first time in *L. pisonis* and the occurrence of kaempferol-3-*O*-rutinoside (13) is also being reported for the first time in the Lecythidaceae family.

Depending on the possible toxic effects of synthetic antioxidants currently used, there is a growing interest in the use of natural products in the pharmaceutical and food industries. Thus, the results suggest that the EtOH extract of the leaves of *L. pisonis* is promising for the development of phytomedicine, cosmeceutical products or adjuvants product where antioxidant activity is desirable.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the financial support and research scholarships. They are also grateful to Dr. Francisco de A. Machado Reis (IQ-UNICAMP) for the NMR analyses, to Dr. Gardene M. Sousa (Herbário Graziela Barroso, (UFPI)) for providing the identification of botanical material and to Professor Cecelia Paz for reviewing the English and grammar.

REFERENCES

Andrade EHA, Zoghbi MDB, Maia JGS (2000). The volatiles from flowers of *Couroupita guianensis* Aubl., *Lecythis usitata* Miers. var. paraensis (Ducke) R. Kunth. and *Eschweilera coriacea* (A. P. DC.) Mori (Lecythidaceae). J. Essent. Oil. Res. 12:163-166.

Braga LF, Sousa MP, Gilberti S, Carvalho MAC (2007). Caracterização morfométrica de sementes de castanha de sapucaia (*Lecythis pisonis* Cambess - Lecythidaceae). Revista de Ciências Agro-Ambientais. 5:111-116.

Brandão MS, Pereira SS, Lima DF, Oliveira JPC, Ferreira ELF, Chaves MH, Almeida FRC (2013). Antinociceptive effect of *Lecythis pisonis* Camb. (Lecythidaceae) in models of acute pain in mice. J. Ethnopharmacol. 146:180-186.

Corrêa MP (1978). *Dicionário das plantas úteis do Brasil e exóticas cultivadas*. Imprensa Nacional: Rio de Janeiro. Brazil p. 777.

Costa DA, Chaves MH, Silva WCS, Costa CLS (2010). Constituintes químicos, fenóis totais e atividade antioxidante de *Sterculia striata* St. Hil. et Naudin. Acta Amazônica 40:207-2012.

Courtois EA, Paine CET, Blandinieres P, Stien D, Bessiere J, Houel, E, Baraloto C, Chave J (2009). Diversity of the Volatile Organic Compounds Emitted by 55 Species of Tropical Trees: a Survey in French Guiana, J. Chem. Ecol. 35:1349-1362.

De-Ekankul W, Potduang B (2003). Biosynthesis of β-sitosterol and stigmasterol in *Croton sublyratus* proceeds via a mixed origin of isoprene units. Phytochem. 62:389-398.

Denadai SMS (2006). Estudo nutricional *in vivo* e *in vitro*, com ênfase em proteínas antinutricionais e tóxicas, de amêndoas de sapucaia (*Lecythis pisonis* Camb.). PhD thesis, Programa Multiinstitucional de Pós-Graduação em Ciências da Saúde- UNB/UFG/UFMS, Campo Grande, Mato Grosso do Sul, Brasil.

Dernovics M, García-Barrera T, Bierla K, Preud'homme H, Lobinski R (2007). Standardless identification of selenocystathionine and its g-glutamyl derivatives in monkeypot nuts by 3D liquid chromatography with ICP-MS detection followed by nanoHPLC–Q-TOF-MS/MS. Analyst. 137:439-449.

Fathiazad F, Delazar A, Amiri R, Sarker SD (2006). Extraction of flavonoids and quantification of rutin from waste tobacco leaves. Iran J. Pharm. Res. 3:222-227.

Junges MJ, Fernandes JB, Vieira PC, Fernandes MFGS, Rodrigues-Filho E, Frühauf M, Barañano AG (2000). Triterpenos ursânicos e oleanânicos isolados do caule de *Eugenia florida* DC. *Revista de Pesquisa e Pós-graduação da Universidade Regional Integrada do Alto Uruguai e das Missões.* 1:13-30.

Mahato SB, Kundu AP (1994). ^{13}C spectra of pentacyclic triterpenoids-a compilation and some salient features. Phytochem. 37:1517-1575.

Mori SA (2001). A família da castanha-do-pará: símbolo do Rio Negro. In: Oliveira AA, Daly DC (eds) Florestas do Rio Negro. Companhia das Letras, São Paulo. p. 337.

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

Oliveira JPC, Ferreira ELF, Chaves MH, Militão GCG, Vieira-Jr GM, Costa AM, Pessoa CO, Moraes MO, Costa-Lotufo LV (2012). Chemical constituents of *Lecythis pisonis* and cytotoxic activity. Rev. Bras. Farmacogn. 22:1140-1144.

Rahman A, Ahmad VU (1992). *^{13}C-NMR of Natural Products: Diterpenes*. Plenum Press, New York, USA. p. 5.

Rufino MSM, Alves RE, Brito ES, Pérez-Jiménez J, Saura-Calixto F, Mancini-Filho J (2010). Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. Food Chem. 121:996-1002.

Salazar GCM, Silva GDF, Duarte LP, Vieira Filho AS, Lula IS (2000). Two epimeric friedelane triterpenes isolated from *Maytenus truncata* Reiss: ^{1}H and ^{13}C chemical shift assignments. Magn. Reson. Chem. 38:977-980.

Silva LL, Gomes BS, Sousa-Neto BP, Oliveira JPC, Ferreira ELF, Chaves MH, Oliveira FA (2012). Effects of *Lecythis pisonis* Camb. (Lecythidaceae) in a mouse modelo of pruritus. J. Ethnopharmacol. 139:90-97.

Sobrinho TJSP, Gomes TLB, Cardoso KCM, Amorim ELC, Albuquerque UP (2010). Otimização de metodologia analítica para o doseamento de flavonoides de *Bauhinia cheilantha* (Bongard) Steudel. Quim. Nova. 33:288-291.

Song N, Xu W, Guan H, Liu X, Wang Y, Nie X (2007). Several flavonoids from *Capsella bursa-pastoris* (L.) Medic. Asian J. Tradit. Med. 2:218-222.

Sousa CMM, Silva HR, Vieira-Jr GM, Ayres MCC, Costa CLS, Araújo DS, Cavalcante LCD, Barros DS, Araújo PBM, Brandão MS, Chaves MH (2007). Fenóis totais e atividade antioxidante de cinco plantas medicinais. Quim. Nova. 30:351-355.