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Phytochemical constituents and preliminary toxicity evaluation of leaves from *Rourea induta* Planch. (Connaraceae)

Milena Kalegari[1*], Marilis Dallarmi Miguel[1], Josiane de Fátima Gaspari Dias[1], Ana Luísa Lacava Lordello[2], Cristina Peitz de Lima[1], Cristina Mayumi Sasaki Miyazaki[1], Sandra Maria Warumby Zanin[1], Maria Christina dos Santos Verdam[1], Obdulio Gomes Miguel[1]

[1]Department of Pharmacy, Federal University of Paraná, [2]Department of Chemistry, Federal University of Paraná

Most active plants are toxic at high doses and it is therefore important to investigate the preliminary toxicity of plant extracts. The *Rourea induta* species is a potential drug with no phytochemical or biological studies registered in the literature. Thus, a phytochemical study and a toxicity analysis of the ethanolic extract obtained from the leaves of *Rourea induta* Planch., Connaraceae, was run. A long chain hydrocarbon, *n*-tetracosane, and four flavonoids were identified: quercetin, and three glycosylated derivates, quercetin-3-*O*-α-arabinofuranoside, quercetin-3-*O*-β-xyloside and quercetin-3-*O*-β-galactoside. This is the first time these have been isolated in this species. The structures were elucidated by ^{13}C NMR, ^{1}H NMR, UV and IR spectroscopy. The toxicity evaluation of extracts was performed by the brine shrimp method and determination of hemolytic activity. The samples demonstrated no toxic potential by the analyzed methods.

Uniterms: *Rourea induta*/phytochemistry. Connaraceae/phytochemistry. Flavonoids. *N*-tetracosane. Plant extracts/toxicity.

A maioria das plantas ativas é tóxica em doses elevadas, portanto, é importante a investigação da toxicidade preliminar dos extratos das plantas. A espécie *Rourea induta* é uma droga potencial que não apresenta estudo fitoquímico ou biológico descrito na literatura. Assim, um estudo fitoquímico e análises toxicológicas foram realizados com o extrato etanólico obtido das folhas de *Rourea induta* Planch., Connaraceae. Foram obtidos um hidrocarboneto de cadeia longa, *n*-tetracosano, e quatro flavonóides, quercetina e três derivados glicosilados, quercetina-3-*O*-α-arabinofuranosideo, quercetina-3-*O*-β-xilosideo e quercetina-3-*O*-β-galactosideo. Esta é a primeira vez que estes compostos são isolados nesta espécie. As estruturas foram elucidadas por espectroscopia de RMN-^{13}C, RMN-^{1}H, ultravioleta e infravermelho. A avaliação da toxicidade dos extratos foi analisada pelo método da *Artemia salina* e atividade hemolítica. Nenhuma das amostras testadas apresentou um potencial tóxico pelos métodos analisados.

Unitermos: *Rourea induta*/fitoquímica. Connaraceae/fitoquímica. Flavonóides. *N*-tetracosano. Extrato de plantas/toxicidade.

INTRODUCTION

The Connaraceae family comprises approximately 16 genera among 300 to 350 species distributed throughout tropical areas of the world; that consists of the angiosperms characterized by ligneous, trees or shrub species. In America, five genera can be found: *Bernardinia, Cnestidium, Connarus, Pseudoconnarus* and *Rourea* (Forero, 2007).

Connaraceae family native species are spread mainly in the Amazon, and the shrubby species are common in "cerrado" (Brazilian ecosystem composed of short trees and shrubs) and are similar to legume species (Lorenzi, Souza, 2005). In "cerrado", the family is represented by seven shrubby-arboreal species distributed around the genera *Rourea* and *Connarus* (Lenza *et al.*, 2008). The cerrado is the second biggest Brazilian and Latin American biome, and has many potential medicinal species that have not yet been subject to scientific study (Recor, 2009).

*Correspondence: M. Kalegari. Departamento de Farmácia, Universidade Federal do Paraná. Av. Prefeito Lothário Meissner, 632 - Jardim Botânico - 80210-170 - Curitiba - PR, Brasil. E-mail: milena.kalegari@gmail.com

Rourea is a pantropical genus and comprises 42 species and 19 varieties; the Amazon is its dispersion center (Forero, 1976; Fonseca, Proença, 2002). The *Rourea induta* Planch. species is characterized by small trees and shrubs that grow to a maximum height of 4 meters. It bears greenish and densely ramified young shrubs. Its cork is widely known for the treatment of Rheumatism (Fonseca, Proença, 2002).

Some important biological activities have been described for species of the Connaraceae family, whereas the *Rourea induta* species has no registered studies in the literature. Phytochemical and biological activity studies are a starting point contributing toward the valuable detection of the species and its isolated compounds.

Most active plants are toxic at high doses and it is thus important to investigate the preliminary toxicity of plant extracts. To achieve a toxicological evaluation, the brine shrimp method and hemolytic activity determination techniques have been employed for different groups (Oliveira *et al.*, 2009). Against this background the aims of this study were to explore and isolate chemical constituents from the ethanol extract of *Rourea induta* Planch., Connaraceae, and to perform a toxicity evaluation of the extract and fractions.

MATERIAL AND METHODS

Plant material

The leaf samples from *R. induta* Planch., Connaraceae were collected in Rondonópolis city, Mato Grosso State, Brazil, in November 2007. The material was identified by Gert Hatschbach, botanist of Curitiba Botanical Museum, Brazil, and the exsiccate was registered under voucher number 261574.

Extraction and isolation

The total amount of ethanol extract was obtained from 2 kg of plant material in 95% ethanol, with the use of Soxhlet equipment, and then filtered, concentrated in a rotary evaporator to 300mL, and fractioned by liquid-liquid partition with solvents of different polarities. From this mixture, hexane, ethyl acetate and chloroform fractions were obtained. The hexane fraction was used to dissolve the filtered residue, and this was fractioned with hexane in a silica column on the Soxhlet equipment. The fractions hexane 1 and hexane 2 were obtained as a result, yielding 5.7834g (0.289%) for hexane 1, 4.9818g (0.249%) for hexane 2, 39.92g (1.99%) for chloroform and 54.3579g (2.717%) for ethyl acetate, at the end of this procedures.

Hexane fraction 1 underwent column chromatography in silica gel stationery phase 60 Merck 0.063 – 0.200 mm, and hexane/ethyl acetate mobile phase at 5% gradient. White crystals (compound **1**)(1.0314 g) were obtained among sub-fractions 1-3.

The chloroform fraction was also submitted to silica column chromatography with a hexane/ethyl acetate eluent system at 5% gradient. It attained ethyl acetate to 100% followed by an ethyl acetate/methanol system at 5% gradient. The samples that had some crystallization were submitted to thin-layer chromatography in mobile phase with ethyl acetate, formic acid, acetic acid, water (100:11:11:27) and reactive NEU (reactive 2-aminoethylbutyrate). A pure compound (compound **2**) (0.1171 g) was identified between sub-fractions 11-16. Sub-fractions 30-36 and 37-44 were submitted to Sephadex LH-20 with 70% methanol as the eluent, and two compounds were obtained denominated **3** (0.0095 g) and **4** (0.022 g).

During the concentration of ethyl acetate fraction, yellow crystals precipitated, which were purified by vacuum filtering and identified as compound **5** (1.5494 g).

Compound identification

The ^{13}C NMR and ^{1}H NMR spectrums were obtained on a Brucker® spectrophotometer model AC200 at 300 MHz. Ultraviolet spectroscopy was carried out on a UV-1601 Shimadzu® spectrophotometer from 200 to 450 nm following the Mabry, Markham & Thomas (1970) methodology. IR spectrums were carried out on pastilles of anhydrous potassium bromide (KBr) compressed on Bomem-Hartmann & Braum MB-series equipment and analyzed on a Biored® FTS 3500 GX, 400 to 4000 cm^{-1}. The melting point was measured on Koffler-Reichert Austria equipment.

***N*-tetracosane (1):** White crystals. mp 65 ºC; IR (KBr) vmax 2918. 2848 (CH_2) cm^{-1}; ^{13}C NMR (CDCl$_3$; 75 MHz) δ: 32.1 (CH_2). 29.9 (CH_2). 29.6 (CH_2). 22.9 (CH_2). 14.3 (CH_3. C-1; C-24); ^{1}H NMR (CDCl$_3$; 300 MHz) 0.8 (3H. *t*); 1.26 (32H. *s*); EIMS *m/z* 338 (5). $C_{24}H_{50}$ (M$^+$). 44 (100) [$C_3H_7^+$]. 57 (95) [$C_4H_9^+$]. 71 (67) [$C_5H_{11}^+$]. 85 (45) [$C_6H_{13}^+$]. 97 (30). 113 (23). 124 (14). 141 (13). 168 (10). 194 (15). 207 (18). 267 (15). 295 (7).

Quercetin (2): Yellow amorphous powder. mp 250 ºC; UV λ$_{max}$ (log ε) (MeOH) 373 (6.32). 256 (6.52). 266(sh) (6.19) nm; (NaOAc) 381(6.28). 325.5 (6.03), 272 (6.24), 256.5(sh) (6.29) nm; (NaOAc/H$_3$BO$_3$) 387.5 (6.39), 260 (6.40) nm; (AlCl$_3$) 458 (6.44), 337 (5.75), 271.4 (6.36) nm; (AlCl$_3$/HCl) 430 (6.34), 363 (5.97), 265.5 (6.38) nm;

IR (KBr) vmax 3406, 3294 (OH), 1664 (C=O), 1610-1400 (C=C) cm^{-1}; ^{13}C NMR (CD$_3$OD; 75 MHz) δ 177.2 (C, C-4), 165.3 (C, C-7), 162.3 (C, C-5), 158.1 (C, C-9), 148.5 (C, C-3'), 147.8 (C, C-2), 146 (C, C-4'), 137.1 (C, C-3), 124 (C, C-1'), 121.6 (CH, C-6'), 116.1 (CH, C-5'), 115.9 (CH, C-2'), 104.1 (C, C-10), 99.1 (CH, C-6), 94.4 (CH, C-8); ^{1}H NMR (CD$_3$OD; 300 MHz) 7.72 (1H, *d*, J=2.1Hz, H-2'), 7.6 (1H, *dd*; J=8.7; 2.1 Hz, H-6'), 6.8 (1H, *d*, J=8.4 Hz, H-5'), 6.36 (1H, *d*, J=2.1 Hz, H-8), 6.17 (1H, *d*, J=2.1 Hz, H-6).

Quercetin-3-*O*-α-arabinofuranoside (3): Yellow amorphous powder, mp 205 °C; UV λ$_{max}$ (log ε) (MeOH) 355 (6.46), 257 (6.57), 267(sh) (6.52) nm; (NaOAc) 370 (6.41), 271 (6.58) nm; (NaOAc/H$_3$BO$_3$) 375 (6.52), 310 (6.10), 261 (6.66) nm; (AlCl$_3$) 436 (6.58), 275 (6.66) nm; (AlCl$_3$/HCl) 400 (6.42), 270 (6.60) nm; IR (KBr) vmax 3427; 3311(OH), 1652 (C=O),1606, 1360 (C=C) cm^{-1}; ^{13}C NMR (CD$_3$OD; 75 MHz) δ 179.9 (C, C-4), 166.0 (C, C-7),163.1 (C, C-5), 159.4 (C, C-9), 158.5 (C, C-2), 149.9 (C, C-3'), 146.4 (C, C-4'), 134.9 (C, C-3), 123.1 (C, C-1'), 122.9 (CH, C-6'), 116.8 (CH, C-2'), 116.4 (CH, C-5'), 109.5 (CH, C-1''), 105.6 (C, C-10), 99.9 (CH, C-6), 94.7 (CH, C-8), 87.9 (CH, C-4''), 83.3 (CH, C-2''), 78.6 (CH, C-3''), 62.5 (CH$_2$, C-5''); ^{1}H NMR (CD$_3$OD; 300 MHz) 7.5 (1H, *d*, J=3.3 Hz, H-2'), 7.4 (1H, *ls*, H-6'), 6.9 (1H, *d,* J=8.4 Hz ,H-5'), 6.38 (1H, *ls* ,H-8), 6.2 (1-H, , *ls,* H-6), 5.58 (1H, *d*, J=1.0 Hz, H-1''), 4.3 (1H, *d*, J=4.3 Hz, H-2''), 3.9 (1H, *dd,* J=4.2; 2.4 Hz, H-3''), 3.8 (1H, *dd,* J=4.2; 8.5 Hz, H-4''), 3.5 (2H, *m,* H-5''a; H-5''b).

Quercetin-3-*O*-β-xyloside (4): Yellow amorphous powder. UV λ$_{max}$ (log ε) (MeOH) 355 (6.65), 257 (6.74) nm; (NaOAc) 371.5 (6.58), 272.5 (6.76) nm; (NaOAc/H$_3$BO$_3$) 375.5 (6.70), 261 (6.83) nm; (AlCl$_3$) 430 (6.68), 273 (6.82) nm; (AlCl$_3$/HCl) 400 (6.60), 270 (6.78)nm; IR (KBr) vmax 3419, 3327 (OH), 1651 (C=O), 1606-1360 (C=C) cm^{-1}; ^{13}C NMR (CD$_3$OD; 75 MHz) δ 179.4 (C, C-4), 166.0 (C, C-7), 163.1 (C, C-5), 158.5 (C, C-2), 158.5 (C, C-9), 149.8 (C, C-3'), 146.1 (C, C-4'), 135.4 (C, C-3), 123.3 (CH, C-6'),123.0 (C, C-1'), 117.2 (CH, C-5'), 115.9 (CH, C-2'), 105.6 (C, C-10), 104.5 (CH, C-1''), 99.8 (CH, C-6), 94.7 (CH, C-8), 77.5 (CH, C-3''), 75.3 (CH, C-2''), 71.0 (CH, C-4''),67.2 (CH$_2$, C-5''); ^{1}H NMR (CD$_3$OD; 300 MHz) 7.6 (1H, H-2'), 7.57 (1H, *d,* J=2.1 Hz, H-6'), 6.84 (1H, *s,* H-5'), 6.39 (1H, *d*, J=1.8 Hz, H-8), 6.16 (1H, *d*, J=2.1 Hz, H-6), 5.17 (1H, *d*, J=7.2 Hz, H-1''), 3.78 (1H, *dd*, J=11.4; 5.0 Hz, H-5''b), 3.46-3.54 (2H, *m*, H-3'' e H-4''), 3.39 (1H, *m,* H-2''), 3.1 (1H, *dd,* J=11.5; 9.5Hz, H-5''a).

Quercetin-3-*O*-β-galactoside (5): Yellow amorphous powder, mp 230 °C; UV λ$_{max}$ (MeOH) 257.9 (6.37),

269(sh) (6.36), 297(sh) (6.06), 359.5 (6.27) nm; (NaOAc) 271.5 (6.30), 372.5 (6.21) nm; (NaOAc/H$_3$BO$_3$) 379.5 (6.32), 262 (6.46) nm; (AlCl$_3$) 272 (6.41), 424.5 (6.22) nm; (AlCl$_3$/HCl) 269 (6.40), 299.5 (sh) (6.00), 400 (6.22) nm; IR (KBr) vmax 3429, 3244 (OH), 1656 (C=O), 1606-1508 (C=C) cm^{-1}; ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 177.5 (C, C-4), 164.2 (C, C-7), 161.3 (C, C-5), 156.2 (C, C-2), 156.3 (C, C-9), 148.5 (C, C-4'), 144.9 (C, C-3'), 133.5 (C, C-3), 122.1 (CH, C-6'), 121.1 (C, C-1'), 115.9 (CH, C-2'), 115.2 (CH, C-5'), 103.9 (C, C-10), 101.8 (CH, C-1''), 98.7 (CH, C-6), 93.5 (CH, C-8), 75.9 (CH, C-5''), 73.2 (CH, C3''), 71.2 (CH, C-2''), 67.9 (CH, C-4''), 60.2 (CH$_2$, C-6''); ^{1}H NMR (300 MHz, DMSO-d$_6$) 12.62 (1H, *s*, 5-OH), 10.87 (1H, *s*, 7-OH), 9.74 (1H, *s*, 4'-OH), 9.17 (1H, *s*, 3'-OH), 7.65 (1H, *dd*, *J*= 8.4 e 2.1 Hz, H-6'), 7.52 (1H, d, *J*= 2.1 Hz, H-2'), 6.82 (1H, *d*, J=8.4 Hz, H-5'), 6.40 (1H,*d,* J=2.1 Hz, H-8), 6.20 (1H,*d*, J=2.1 Hz, H-6), 5.38 (1H, *d*, *J*=8.4 Hz, H-1''), 5.14 (1H, *s*, 2''-OH), 4.87 (1H, *s*, 3''-OH), 4.44 (2H, *s,* 4'', 6'' – OH).

Brine shrimp bioassay

The brine shrimp (*Artemia salina*) method was first described by Meyer *et al.* (1982). The eggs of brine shrimp hatched within 48 hours in artificial sea water, producing a large number of larvae. The samples, ethanolic extract, and all the fractions, were tested at concentrations of 1000, 100 and 10 μg/mL. Ten shrimps were transferred to the samples and after 24 hours survivors were counted and percentage of deaths at each dose recorded. Quinidine sulfate was used as a positive control and the solvent used to prepare the samples as the negative control. The test was run in triplicate and the data was analyzed by the Probitos method to determine LD$_{50}$ with a 95% confidence interval.

Hemolytic activity

Ethanolic extract and fractions were submitted to hemolytic activity tests. The first method tested was based on the WHO (1998) and Carvalho *et al.* (2009). For this method, a 2% sheep red blood cell suspension (Newprov®) was prepared in pH 7.4 phosphate buffer. The samples were diluted in 10% ethanol with NaCl 0.9% and tested at concentrations of 1000, 500, 200 and 100 μg/mL. A volume of 1.0 mL of the blood suspension was added to the samples which were then mixed slowly and left to rest for 30 minutes, mixed again and kept at rest for a further 150 minutes. Subsequently, the samples were centrifuged for 5 minutes at 3000 rpm. Distilled water was used as a positive control and solvent and phosphate buffer as a negative control. The result was reported as presence or

absence of hemolysis based on the supernatant tonality after centrifugation. The presence of a red blood cell precipitate indicated a negative result.

The second method tested was agar diffusion using plates with sheep red blood cell agar (Newprov®). The samples were prepared at 1000 μg/mL and impregnated in sterile discs (Whatmann nº1, diameter 7 mm). After solvent evaporation, the discs were distributed in the plates, which were incubated at 36 °C for 24 hours. Hemolytic halos were then measured and results expressed as mean halos found in duplicate. Discs with the solvents used for sample dilution were used as a negative control and Triton X-100 at 1000μg/mL as a positive control (Efing, 2008).

RESULTS AND DISCUSSION

The hexane fraction of ethanol extract from the leaves of *R. induta* was subjected to a chromatographic step over silica gel to yield compound **1** (n-tetracosane), while the chloroform fraction was subject to multiple chromatographic steps over silica gel to yield compounds **2**, **3** and **4** (quercetin, quercetin-3-*O*-α-arabinofuranoside and quercetin-3-*O*-β-xyloside, respectively). Compound **5** (quercetin-3-*O*-β-galactoside) was obtained during ethyl acetate fraction concentration.

The ^{1}H NMR signals for compound **1**, in the range 0.8 – 1.7ppm, indicated a region characteristic of aliphatic alicyclic compounds with three hydrogen at δ0.8, whereas the signal integration at δ1.26 indicated about 32 hydrogen, corresponding to CH_2. The ^{13}C NMR spectrum characterized five signals in the range 14- 32ppm. The mass spectrum showed a characteristic fragmentation of aliphatic hydrocarbons, with 14 mass units between peaks. The fragmentation standard is determined by C-C fragmentation because hydride (H-) elimination is difficult. The spectrum showed higher intensity peaks (44 and 57) that represented the fragments with three and four carbons, and a low intensity molecular ion (*m/z*) of 338, characteristic of long chain. The melting point was 65 °C a typical value for a hydrocarbon. These results were in line with those of Siddiqui *et al.* (2004) and denoted that compound **1** was n-tetracosane ($C_{24}H_{50}$).

For compounds **2-5**, the UV spectrum showed typical results for flavonoids, with bands between 256-259 nm relative to ring A and 348-360 relative to ring B (Mabry, Markham, Thomas, 1970), which suffers a hypsochromic effect in comparison to quercetin due to C-3 substitution for compounds **3**, **4** and **5** (Santos, Schripsema, Kuster, 2005). For all samples, the addition of NaOAc produced a bathochromic effect of band II, which indicated a free hydroxyl in position 7. H_3BO_3 led to a bathochromic shift

of band I in the presence of an orthodihydroxyl group in ring B. For **3**, **4** and **5**, the spectrum with HCl revealed a bathochromic shift for bands I and II. The addition of $AlCl_3$ caused a bathochromic effect of band I between 35-55nm compared with methanol spectrum, typically shifted for 5-hydroxyflavons with position 3 replaced. For compound **2** this effect was 57nm, a typical result for 3,5-hydroxyflavons (Mabry, Markham, Thomas, 1970).

The infrared spectrum revealed two absorption bands in 3430 and 3300 cm^{-1} that typically result from the OH link axial deformation. Moreover, the existence of an aromatic ring may be observed, evidenced by a set of bands that range between 1600 and 1400 cm^{-1}, which denotes the axial deformation of the aromatic C=C. Bands close to 1650 cm^{-1} suggest the presence of carbonyl (Silverstein, Bassler, Morril, 1994).

The ^{13}C NMR signals for these compounds were in good agreement with those of quercetin, the difference between them was located at the glycoside linked to the C-3 of the aglycone. This glycoside was identified by the signals at sugar region (60-80ppm) in the spectrum by comparing against those described in the literature. For compound **2,** no signals were identified at this region (Andersen, Markham, 2006). The signals for compound **3** indicated arabinofuranoside (Vvdenskaya *et al.*, 2004), for compound **4** xyloside (Yan *et al.*, 2002) and for the isolated **5** galactoside (Jiang *et al.*, 1990). The ^{1}H NMR signals for these compounds were found in the aromatic region (6-8ppm) and sugar region (3-4.2 ppm). The configuration of the sugars were determined by the coupling constant of the anomeric hydrogen, where compound **3** showed the anomeric hydrogen at δ5.58 with J=1.00 Hz, indicating that arabinofuranoside is α (Zhang *et al.*, 2005), compound **4** was δ5.17 with J=7.2 Hz, showing a β configuration for xyloside (Yan *et al.*, 2002), and compound **5** was δ5.39 with J=7.8 Hz, a β configuration for galactoside (Santos, Schripsema, Kuster, 2005).

After the above analysis the results were compared against literature data (Tables I, II and III) and compound

TABLE I - Compound 1 NMR (ppm) experimental data compared against literature data

Experimental [13]C	Siddiqui *et al.* (2004) [13]C
32.1	32.1
29.9	29.8
29.6	29.5
22.9	22.8
14.3	14.1

TABLE II - Compounds 2 and 3 NMR (ppm) experimental data compared against literature data

Position	Quercetin			Quercetin-3-*O*-α-arabinofuranoside			
	Experimental		Andersen, Markham (2006)	Experimental		Lhuillier (2007)	
	^{13}C	^1H	^{13}C	^{13}C	^1H	^{13}C	^1H
2	147.8	-	148.00	158.5	-	158.2	-
3	137.1	-	137.21	134.9	-	133.7	-
4	177.2	-	177.33	179.9	-	178.8	-
5	162.3	-	162.50	163.1	-	161.8	-
6	99.1	6.17 *d* (*J*=2.1 Hz)	99.25	99.9	6.2 *ls*	98.9	6.21*d* (*J*=2.0 Hz)
7	165.3		165.34	166.0	-	164.8	-
8	94.4	6.36 *d* (*J*=2.1 Hz)	94.40	94.7	6.38 *ls*	93.5	6.4 *d* (*J*=2.0Hz)
9	158.1	-	158.22	159.4	-	157.4	-
10	104.1	-	104.52	105.6	-	104.4	-
1'	124.0	-	124.15	123.1	-	121.9	-
2'	115.9	7.72 *d* (*J*=2.1 Hz)	115.99	116.8	7.5 *d* (*J*=3.3 Hz)	115.7	7.54*d* (*J*=2.1 Hz)
3'	148.5	-	148.75	149.9	-	148.7	-
4'	146.0	-	146.21	146.4	-	145.1	-
5'	116.1	6.8 *d* (*J*=8.4 Hz)	116.22	116.4	6.9 *d* (*J*=8.4 Hz)	115.3	6.92*d* (*J*=8.3 Hz)
6'	121.6	7.6 *dd* (*J*=8.7; 2.1 Hz)	121.67	122.9	7.4 *ls*	121.8	7.5*dd* (*J*=8.3 e 2.1 Hz)
				Arabinofuranoside			
1''	-	-	-	109.5	5.5 *s*	108.5	5.48*d* (*J*=1.0Hz)
2''	-	-	-	83.3	4.3 *d* (*J*=2.4Hz)	82.1	4.35*dd* (*J*=3.0; 1.0Hz)
3''	-	-	-	78.6	3.9 *dd* (J=4.2; 2.4Hz)	77.5	3.93*dd* (*J*=5.0; 3.0Hz)
4''	-	-	-	87.9	3.8 *dd* (J=4.2; 8.5Hz)	86.8	3.89 *m*
5''	-	-	-	62.5	3.5 m (2H)	61.4	3.52*dd* (*J*=11.5; 4.0Hz) 3.48*dd* (*J*=11.5; 3.6Hz)

Note: *ls*= large singlet, *d*=doublet, *dd*= double doublet, *m*= multiplet, *J*= coupling constant

2 was identified as quercetin (Andersen, Markham, 2006), **3** as quercetin-3-*O*-α-arabinofuranoside (Vvdenskaya *et al.*, 2004; Lhuillier, 2007), **4** as quercetin-3-*O*-β-xyloside (Yan *et al.*, 2002), and **5** as quercetin-3-*O*-β-galactoside (Jiang *et al.*, 1990), also known as hyperin (Figure 1).

Quercetin and quercetin-3-*O*-α-arabinofuranoside were previously isolated from the Connaraceae family spe-

cies *Byrsocarpus coccineus* (Ahamadu *et al.*, 2007), while hyperin was previously isolated in the *Rourea microphylla* species (Jiang *et al.*, 1990). However, this is the first time these have been isolated in the *R. induta* species. There are no previous reports on the isolation of quercetin-3-*O*-β-xyloside in the Connaraceae family.

The toxicity evaluation against *Artemia salina*

TABLE III - Compounds 4 and 5 NMR (ppm) experimental data compared against literature data

Position	Quercetin-3-O-xyloside				Quercetin-3-O-β-galactoside			
	Experimental		Yan *et al.* (2002)		Experimental		Jiang *et al.* (1990)	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
2	158.5	-	152.0	-	156.2	-	156.28	-
3	135.4	-	130.0	-	133.5	-	133.61	-
4	179.4	-	179.5	-	177.5	-	177.44	-
5	163.1	-	162.0	-	161.3	-	161.15	-
6	99.8	6.16 *d* (*J*=2.1 Hz)	100.1	6.20	98.7	6.2 *d* (*J*=1.8 Hz)	98.62	6.20 *s*
7	166.0	-	166.3	-	164.2	-	164.02	-
8	94.7	6.39 *d* (*J*=1.8 Hz)	94.9	6.39	93.5	6.4 *d* (*J*=2.1 Hz)	93.45	6.41 *s*
9	158.5	-	158.6	-	156.3	-	156.28	-
10	105.6	-	100.1	-	103.9	-	103.94	-
1'	123.0	-	123.4	-	121.1	-	121.16	-
2'	115.9	7.6	116.2	7.6	115.9	7.5 *d* (*J*=2.4 Hz)	115.17	7.53 *s*
3'	149.8	-	143.0	-	144.9	-	144.67	-
4'	146.1	-	146.0	-	148.5	-	148.32	-
5'	117.2	6.84 *s*	117.4	6.85	115.2	6.8 *d* (*J*=8.7 Hz)	116.10	6.82 *d* (*J*=8 Hz)
6'	123.3	7.57 *d* (*J*=2.1 Hz)	123.2	7.57	122.1	7.65 *dd* (*J*=8.4; 2.1 Hz)	121.62	7.67 *d* (*J*=8 Hz)
	Xylose				Galactoside			
1''	104.5	5.17 *d* (*J*=7.2 Hz)	104.7	5.18 *d* (*J*=6 Hz)	101.8	5.39 *d* (*J*=7.8 Hz)	102.12	
2''	75.3	3.39 *m*	75.4	3.4 *d*	71.2	3.55 *dt* (*J*=7.8; 3.9 Hz)	71.26	
3''	77.5	3.46-3.54	77.7	3.34 s	73.2	3.33	73.27	
4''	71.0	3.46-3.54	71.2	3.45-3.54 *m*	67.9	3.64 *ls*	67.93	
5''	67.2	3.1 *dd* (*J*=11.5; 9.5 Hz) 3.78 *dd* (*J*=11.4; 5.0 Hz)	67.4	3.10 *dd* 3.76 *dd*	75.9	3.30 *s*	75.73	
6''	-	-	-	-	60.2	3.25 e 3.45 (2H)	60.14	

Note: *s*= singlet; *ls*= large singlet, *d*=doublet, *dd*= double doublet, *dt* = double triplet, *m*= multiplet, *J*= coupling constant

(Table IV) demonstrated that all the samples showed a median lethal concentration greater than 1000 µg/mL, the maximum concentration for a sample to be considered active (Meyer *et al.*, 1982).

This bioassay is used to evaluate the acute toxicity and is considered essential as a preliminary assay in the study of compounds with potential biological activities (Amaral, Silva, 2008). Analyzing the results, it can be concluded that neither the ethanolic extract, nor the fractions from *R. induta*

exhibited toxic potential against the brine shrimp.

For the hemolytic tests, the tested sample showed no hemolytic ability according to the two methods analyzed. In the test using the red blood cell suspension, none of the supernatants showed red coloration and all had a red blood cell precipitate at the tested doses and therefore caused no hemolysis in red blood cells. This result indicates that neither the fractions nor the ethanolic extract are toxic against red blood cells. These results were confirmed by the agar

TABLE IV – *Artemia salina* lethality and LD_{50}

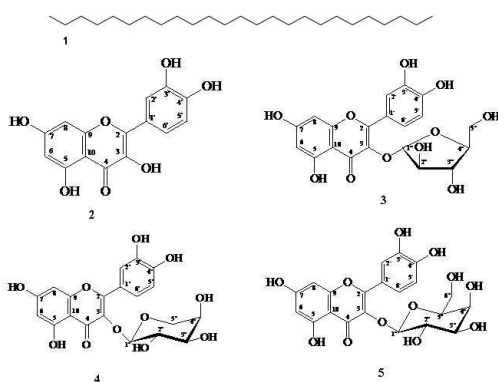| Sample	Mortality/Concentration			LD_{50} (µg/mL)	95% Confidence interval (µg/mL)
	10 (µg/mL)	100 (µg/mL)	1000 (µg/mL)		
Ethanolic extract	1	1	4	>1000	–
Fraction Hexane 1	0	0	0	> 1000	–
Fraction Hexane 2	0	1	4	> 1000	–
Fraction Chloroform	0	0	0	> 1000	–
Fraction Ethyl Acetate	0	0	1	> 1000	–
Quinidine sulfate	16	10	18	50.12	35.80-70.16

FIGURE 1- Chemical structures of compounds **1-5**.

diffusion method, on which none of the samples had a hemolytic halo around the impregnated disc, confirming the absence of hemolytic activity. Similarly, the negative controls exhibited no hemolytic halo, whereas the Triton X-100 had a halo of 2 cm.

Red blood cell lyses can cause an elevation in plasma hemoglobin leading to harmful effects in the kidneys (nephrotoxicity), and in the cardiovascular system (vasomotor effect) (Carvalho *et al*., 2007). Therefore, negative hemolytic activity is a good indicator because it confirms no toxicity to erythrocyte membrane.

The acute toxicity results showed that the *Rourea induta* extracts had no toxic potential, thus favoring the species for further study such as pharmacological analyses, to determine therapeutic properties of this species.

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