Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **28** (2): 234-40 (2009)

Original Article

Received: August 26, 2008
Accepted: January 20, 2009

Screening of Cytotoxic Activity in Hexanic and Ethanolic Extracts of *Rollinia laurifolia*

M.M. CUNHA [1], F.C. NASCIMENTO [3], L.P. SANTOS PIMENTA [4], M.A.D. BOAVENTURA [4] , C.E. SALAS [2] & M.T.P. LOPES [1]*

[1] *Departamento de Farmacologia,*
[2] *Departamento de Bioquímica e Imunologia -ICB,*
Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, CEP 31270-901, Belo Horizonte, MG, Brazil.
[3] *Instituto de Química, Campus do Paricarana, UFRR, CEP 69301-270, Boa Vista, RR, Brazil*
[4] *Departamento de Química – ICEx – Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, CEP 31270-901, Belo Horizonte, MG, Brazil.*

SUMMARY. The hexanic and ethanolic extracts obtained from *Rollinia laurifolia,* collected in Minas Gerais State/Brazil, were screened by the brine shrimp lethality and cytotoxic assays on different cell lines, in order to identify potential sources for novel antitumoral compounds. Fifty percent of the ethanolic derived fractions (E) showed significant cytotoxic activity ($IC_{50} < 10^{-1}\mu g/mL$), not related to tannins or saponins, i. e., IC_{50} E13 on $C_6 = 1.54 \times 10^{-2}$ $\mu g/mL$, IC_{50} E29 on L_{929} and $C_6 = 6.43 \times 10^{-2}$ $\mu g/mL$ and 3.57×10^{-2} $\mu g/mL$, respectively. RP-HPLC analysis led to the purification and identification of two known acetogenins, gonionenin and annonin-I, detected for the first time in *Rollinia sp,* and a new acetogenin, named *laurifolin*.

INTRODUCTION

Plants from *Annonaceae* family are very important sources of edible fruits and extracts for fragrances. A special attention is given to their uses in folk medicine for its alleged properties as: antitumor, antiparasitic and antidiarrheic [1,2]. From a phytochemical viewpoint, plants from this family have been intensively investigated, initially motivated by the isolation of numerous alkaloids [2] and, more recently, due to the presence of annonaceous acetogenins [3]. The number of studies on these substances has increased in the last decade due to their broad range of potential bioactivities, as cytotoxic, antitumoral, pesticidal, among others [3]. Their effect is believed to be associated to a decrease in ATP production by inhibiting mitochondrial NADH:ubiquinone oxidoreductase and a plasma membrane ubiquinone NADH oxidase [4,5]. Only six *Rollinia* species have been already studied, contributing with new specific annonaceous acetogenins [6]. *Rollinia laurifolia* Schldtl, the subject of this report, is a native tree from the Brazilian "cerrado". The hexanic leaf extract of *R. laurifolia* led to isolation of a new acetogenin laurifolin (Fig. 1-1) and one known acetogenin - gonionenin (Fig. 1-2) [7,8], while the ethanolic extract yielded the known acetogenin annonin I (Fig. 1-3) [9].

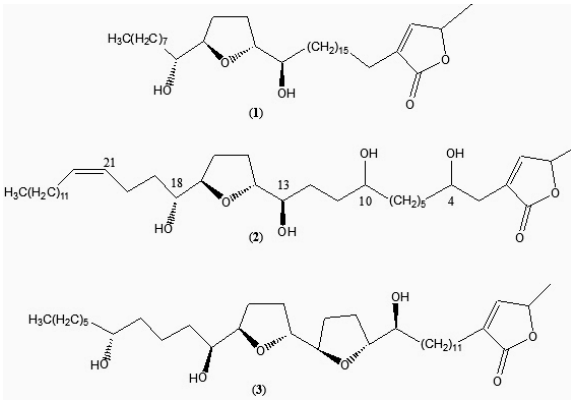

Figure 1. Structures of annonaceous acetogenins isolated from bioactive fractions of hexanic or ethanolic leaf extract of *Rollinia laurifolia*. (**1**) laurilfolin; (**2**) gonionenin, (**3**) annonin I.

KEY WORDS: Annonaceae, Acetogenins, Cytotoxic activity, *Rollinia laurifolia*.

* Author to whom correspondence shoud be addressed. *E-mail:* mtpl@icb.ufmg.br

ISSN 0326-2383

We report here the cytotoxic activity displayed by hexanic and ethanolic leaf fractions and/or purified substances derived from these fractions from *Rollinia laurifolia*.

EXPERIMENTAL

Plant material

Leaves of *Rollinia laurifolia* Schldtl were collected on the campus of Universidade Federal de Minas Gerais, voucher specimen (BHCB N° 22749), in August of 1996. The leaves were dried at 40 °C, pulverized and extracted at room temperature with hexane followed by ethanol affording the hexanic (RLHF 2.3% dry wt) and ethanolic extracts (RLEF 6.0% dry wt).

Chromatographic analysis

Each extract and derived fractions were subjected to TLC analysis. The plates were sprayed with Kedde's Reagent which provides a positive test to identify the α,β-unsaturated-γ-lactone moiety commonly found in annonaceous acetogenins [10]. Each extract and derived fractions were developed following TLC by spraying and with Dragendorff's Reagent, as well. Each of the samples studied gave positive reaction, evidencing the presence of alkaloids and/or acetogenins [11].

The hexanic extract (68.0 g) was further fractionated on a silica gel column with a succession of solvents of increasing polarity, composed by C_6H_6 / CH_2Cl_2 / AcOEt / MeOH, affording 320 fractions that, after TLC analysis, were pooled into 14 fractions. Three of them, that tested positive for Kedde's reagent and showed IR absorption at 1745 cm^{-1} featured by the unsaturated γ–lactone present in acetogenins, were chosen for further purification. These fractions named H9, H11 and H14, were partitioned with hexane and MeOH-H2O (10%) to yield the hydroalcoholic and hexanic phases. The hydroalcoholic residues were chromatographed by reverse phase MPLC and Sephadex LH-20 yielding: H9.1, H11.12, H11.13, H14.2, H14.3, H14.4 and H14.5. Fraction H9.1 was subjected to repeated purification cycles in RP-18 HPLC (isocratic elution, MeOH-H_2O, 85:15) to yield the known acetogenin goniothenin (Fig. 1). H11.12 was purified by reverse phase MPLC, followed by RP-18 HPLC (isocratic elution, MeOH-H_2O-THF, 90:0.5:0.5) to yield the acetogenin, laurifolin (Fig. 1) and fraction H11.13 characterized as a mixture of annonaceous acetogenins containing a novel compound named rolilaurin and two previously characterized substances, uvariamicin-I and uvariamicin-II. Fractions H14.2, H14.3, H14.4 and H14.5 after several steps of purification failed to resolve a single species and were also characterized as a mixture of annonaceous acetogenins. The ethanolic extract (58.0 g) was eluted on a polyamide column (0.07 mm, 191.0 g, Machereys, Nagel) with AcOEt followed by MeOH. The methanolic fraction (43.0 g) was subsequently fractionated on a silica-gel column with a sequential gradient composed by C_6H_6/CH_2Cl_2/AcOEt/MeOH, yielded 440 fractions which upon identification as acetogenins by TLC, were pooled into 39 fractions. Fractions E13, E27, E29 and E31, were further characterized as a mixture of annonaceous acetogenins by TLC, IR spectra and RMN ^1H. Fraction E13 was further resolved by reverse phase HPLC yielding the known acetogenin annonin I (squamocin) (Figure 1). The remaining fractions were not further studied because of their limited amounts available.

Biological screening

Brine shrimp lethality assay [12]

Artemia salina encysted eggs *(A. franciscana),* gift from Dr. Eupídio Beltrani - Laboratório de Camarões, UFSC, were incubated in seawater under artificial lighting at 28 °C, pH 7-8. After 24 h, nauplii were collected and kept for additional 24 h under the same conditions to attain the metanauplii stage. The assayed samples (triplicate) were dissolved in DMSO (dimethylsulfoxide) (1 mg/mL) and serially diluted in seawater. About 10-20 nauplii were added to each set of tubes containing the samples. To verify the solvent effect 50 µL of DMSO were assayed in the presence of seawater. A positive or negative control dissolved in seawater included lapachol (1 mg/ml) and rutin (1 mg/ml), respectively. After a 24 h period, the number of survivors was counted and the 50% lethal concentration (LC_{50}) with a 95% confidence interval was calculated by Probit analysis [13].

Qualitative analysis [14]

Tannins and/or polyphenols

The assayed samples (10 µL), at their LC_{50} were applied onto a punctured 0.1% gelatin gel containing 1.5% agar plated on Petri dishes. The resultant protein precipitation halo was measured after 24 h. The samples or the positive control containing 10 mg/ml tannic acid were dissolved in DMSO before application.

Saponins

Holtzman rat erythrocytes were obtained by cardiac puncture in the presence of 3.8% sodium citrate. The blood was centrifuged for 5 min at 2,000 x rpm and the pellet rinsed 3 times with saline isotonic solution. The erythrocytes were mixed with 1.3% agar suspension and plated on Petri dishes. The samples were applied onto the agar as described earlier for tannins and/or polyphenols. Following a 24 h period, the hemolytic halo was measured. Both, samples and the saponin positive control, used to calibrate a standard curve (0.5-2.0 mg/ml) were dissolved in DMSO.

Cell lines

The cell lines were kindly supplied by Dr. H. Armelin (Instituto de Química - USP-Brasil): chinese hamster ovary (CHO), mouse tumorigenic fibroblast (L929) and rat glioma (C6). Besides, Dr. R.R. Brentani (Ludwig Institute for Cancer Research-São Paulo-Brasil) supplied: human melanoma (Mewo) and human breast carcinoma (MDA MB 231). The cells were maintained as monolayer culture in RPMI 1640 medium supplemented with 5% heat-inactivated fetal bovine serum (FBS) and antibiotics at 37 °C in 3% CO_2. Cells were grown to 80-90% confluence and released using 0.2% trypsin solution.

MTT assay [15]

The viable cells counted by trypan blue exclusion staining assay (3 x 10^3 / well) were plated onto 96-well microplates containing 5% FBS-RPMI 1640 medium and incubated at 37 °C in 3% CO_2. Following 24 h incubation period, the test samples diluted to 0.1% (v/v) in ethanol were added and the spectrometric readings (600 nm) performed at 24, 48, 72 and 96 h after the sample addition. A solution of tetrazolium salt (5.0 mg/mL) (Sigma Chem. Co.) was added to cultures 4 h before the readings. The 50% inhibitory concentration (IC_{50}) was estimated by linear regression of at least four triplicate concentrations.

RESULTS

In this study two leaf extracts, and several fractions derived from them, obtained from *R. laurifolia* were assayed for their cytotoxic activity.

Cytotoxic activity of the methanolic fractions from hexanic extract

Initially, the toxicity on *A. salina* using LC_{50} and the presence of tannins or saponins in fractions derived from the hexanic extract was evaluated (Table 1). LC_{50} values between 9.3 (H11.13) to 25 µg/mL (H11.12) were observed while the positive control lapachol displayed a LC_{50} = 16.7 µg/mL. The chemical tests showed the absence of tannins, while saponins were absent in about 80% of the analyzed fractions.

Since every fraction yields an LC_{50} value similar to the lapachol control, and due to the reported presence of annonaceous acetogenins, the fraction selection for the cytotoxic test was based on the identification of acetogenins by TLC, IR spectra and yield of each fraction.

Table 2 summarizes the IC_{50} induced by the selected fractions in a tumor panel composed of MDA MB 231, L929, Mewo and C6 cell lines. Fraction H9.1 exhibited low cytotoxic activity on tumoral cell lines (MeWo – 2.81 µg/mL, MDA MB-231 – 4.03 µg/mL, C6 – 4.87 µg/mL and L929 – 7.70 µg/mL). This fraction was subjected to repeated chromatographic steps yielding the acetogenin gonionenin (Fig. 1-2) previously isolated in *Goniothalamus giganteus* [16], but detected for the first time in the genus *Rollinia* [8].

Fraction H14 was subjected to chromatography on silica gel yielding fractions H14.2, H14.3,

Fraction	LC_{50} µg x 10^1 /ml	Tannins and/or poliphenols	Saponins
H9	1.52 ± 0.20	Negative	Negative
H11.12	2.53 ± 0.20	Negative	Negative
H11.13	0.93 ± 0.04	Negative	Negative
H14.2	1.20 ± 0.06	Negative	Negative
H14.3	1.70 ± 0.04	Negative	Positive
H14.4	1.05 ± 0.53	Negative	Negative
H14.5	1.07 ± 0.11	Negative	Negative

Table 1. Toxicity on *Artemia salina* of hexane fractions from *Rollinia laurifolia* leaves. H represents the hexanic fraction. Lapachol Positive Control – LC_{50}: = 1.67 µg x 10^1/mL, Rutin Negative Control > 50 x 10^1 µg/mL.

Fraction	IC_{50} µg/mL (95% confidence interval)			
	Tumoral cell lines			
	MDA MB 231	**L929**	**MeWo**	**C6**
H9.1	4.03 (2.97 - 5.54)	7.70 (3.35 - $2.11x10^{1}$)	2.81 ($2.52x10^{-1}$ - $3.49x10^{1}$)	4.87 (1.26 - $1.91x10^{1}$)
H11.12	1.84 ($5.24x10^{-1}$ - 6.31)	4.06 (4.49 - 4.77)	3.40 ($1.36x10^{-1}$ - $8.78x10^{1}$)	$1.62x10^{-1}$ ($4.68x10^{-4}$ - 1.43)
H11.13	3.72 (2.24 - 6.82)	$<2.0x10^{-1}$	2.56 ($1.41x10^{-1}$ - $4.74x10^{1}$)	ND
H14.2	ND	ND	$1.10x10^{2}$ ($1.98x10^{-7}$ - $4.86x10^{14}$)	ND
H14.3	$1.81x10^{1}$ (2.35 - $1.69x10^{2}$)	$1.89x10^{2}$ ($2.69x10^{-8}$ - $1.07x10^{15}$)	$2.11x10^{1}$ ($3.99x10^{-2}$ - $3.22x10^{4}$)	ND
H14.4	$4.35x10^{1}$ ($1.04x10^{1}$ - 3.73 $x10^{2}$)	$4.75x10^{1}$ ($2.13x10^{-3}$ - $6.69x10^{6}$)	$2.08x10^{2}$ ($1.34x10^{2}$ - $3.16x10^{3}$)	$4.14x10^{3}$ ($1.40x10^{2}$ - $1.70x10^{12}$)
H14.5	$7.42x10^{1}$ ($4.21x10^{-2}$ - $8.82x10^{5}$)	$1.44x10^{3}$ ($1.78x10^{2}$ - $8.52x10^{4}$)	$1.82x10^{1}$ ($1.11x10^{1}$ - $3.02x10^{1}$)	ND
crassiflorin	$1.23x10^{-2}$ ($5.73x10^{-3}$ - $3.5x10^{-2}$)	ND	ND	$5.19x10^{-2}$ ($1.62x10^{-2}$ - $4.04x10^{-1}$)
mitomycin	$4.56x10^{-3}$ ($2.52x10^{-3}$ - $9.46x10^{-3}$)	$2.98x10^{-2}$ ($1.45x10^{-2}$ - $7.87x10^{-2}$)	$2.85x10^{-2}$ ($1.85x10^{-2}$ - $4.27X10^{-2}$)	$4.00x10^{-4}$ ($3.43x10^{-4}$ - $4.68x10^{-4}$)

Table 2. Cytotoxicity on tumoral cell lines of hexane fractions from *Rollinia laurifolia* leaves.

H14.4 and H14.5 that afforded a positive reaction with Keed's reagent for acetogenins and featured characteristic signals on ^{1}H NMR and IR spectra. These fractions also showed low activity on the tumor cell panel, attaining IC_{50} values higher than 10 µg/mL.

By contrast, fractions H11.12 and H11.13 depicted lower IC_{50} on C6 ($1.62x10^{-1}$ µg/mL) and L929 cells ($<2.0x10^{-1}$ µg/mL), respectively. These two fractions did not present a strong inhibitory effect on the others cell lines (IC_{50} 1.84 to 4.06 µg/mL), but fraction H11.12 was cytotoxic (IC_{50} = $5.40x10^{-1}$ µg/mL) on the normal CHO cell line, as well (data not shown). This fraction was further purified by reverse phase HPLC yielding the recently described acetogenin, laurifolin (Fig. 1-1) [7].

Cytotoxic activity of methanolic fractions from ethanolic extract

As previously shown for the hexanic extract, the LC_{50} and the presence of tannins and saponins was also investigated in the methanolic fractions separated from ethanolic leaf extracts of *R. laurifolia*. The data from these experiments are summarized on Table 3. The observed LC_{50} ranged between 0.04 to $4.13x10^{1}$ µg/mL. Qualitative tests used to identify tannins or saponins failed to detect any of these substances in about 85% of analyzed fractions.

As before, since each fraction afforded cytotoxicity comparable to the positive control, we selected those fractions with higher yield and fewer components for further purification. In Table 4 are summarized the IC_{50} of these fractions on tumoral cell lines. Because of the limiting amounts of E13, its cytotoxicity was only evaluated on L929 and C6 cells. The inhibitory effect of this fraction was strong against C6 (IC_{50}=1.54x10-2 µg/mL) but marginal against L929 cells. Analysis by ^{1}H- and ^{13}C- NMR spectral data of E13 revealed a mixture of acetogenins. This fraction was subjected to RP-HPLC to yield the pure acetogenin annonin I (Fig. 1-3), isolated before in *Annona squamosa* 17, and reported for the first time in the genus *Rollinia* [9].

The activity of E27 was moderate towards C6 (IC_{50}=1.58 µg/mL) and MeWo (8.40 µg/mL), but lower towards MDA MB-231 cells (13.10 µg/mL). By contrary, fraction E29 exhibited a strong effect in most cells with the exception of MDA MB 231 (IC_{50}=3.17 µg/mL). The higher IC_{50} values were found in L929 and C6

Fraction	LC_{50} µg x 10^1 /ml	Tannins and/or poliphenols	Saponins
E13	< 0.70	*	Negative
E23	0.80 ± 0.14	Negative	Positive
E24	< 0.66	Negative	Negative
E27	< 0.43	Negative	Negative
E28	0.21 ± 0.01	Negative	Negative
E29	0.32	Negative	Negative
E31	< 0.04	Negative	Negative
E32	4.13 ± 0.46	NT	Negative
E33	1.44 ± 0.63	Negative	Negative
E34	2.83 ± 0.30	Positive	Negative
E35	2.80 ± 0.35	Negative	Negative
E38	2.05 ± 0.35	Negative	Negative

Table 3. Toxiciy on *Artemia salina* of ethanol fractions from *Rollinia laurifolia* leaves. NT = not tested. E = denotes an ethanol fraction. Lapachol Positive Control – LC_{50}: = 1.67 µg x 10^1/mL, Rutin Negative Control > 50 x 10^1 µg/mL.

Fraction	IC_{50} µg/mL (95% confidence interval)			
	Tumoral cell lines			
	MDA MB 231	L92	MeWo	C6
E13	NT	3.57 ($6.53x10^{-1}$ - $1.75x10^1$)	NT	$1.54x10^{-2}$ ($4.39x10^{-3}$ - $3.98x10^{-2}$)
E27	$1.31x10^1$ (2.56 - $7.07x10^1$)	ND	8.40 ($5.94x10^{-1}$ - $1.21x10^2$)	1.58 ($1.05x10^{-1}$ - $2.35x10^1$)
E29	3.17 ($2.33x10^{-2}$ - $8.78x10^3$)	$6.43x10^{-2}$ ($3.18x10^{-2}$ - 1.10 $x10^{-1}$)	$1.07x10^{-1}$ ($4.53x10^{-4}$ - 9.25)	$3.57x10^{-2}$ ($3.02x10^{-4}$ - $1.81x10^{-1}$)
E31	2.61 ($3.54x10^{-1}$ - $2,06x10^1$)	$2.67x10^{-1}$ (7.39x10-3 - 7.86)	$1.50x10^{-1}$ ($9.56x10^{-6}$ - $7.77x10^{-1}$)	ND
E31.1	$1.67x10^1$ ($4.48x10^{-1}$ - $1.02x10^2$)	1.44 $x10^3$ ($1.78x10^2$ - $8.52x10^4$)	$1.82x10^1$ ($1.11x10^1$ - $3.02x10^1$)	1.93 ($3.17x10^{-1}$ - $1.16x10^1$)
Crassiflorin	$1.23x10^{-2}$ ($5.73x10^{-3}$ - $3.5x10^{-2}$)	ND	ND	$5.19x10^{-2}$ ($1.62x10^{-2}$ - $4.04x10^{-1}$)
Mitomycin	$4.56x10^{-3}$ ($2.52x10^{-3}$ - $9.46x10^{-3}$)	$2.98x10^{-2}$ ($1.45x10^{-2}$ - $7.87x10^{-2}$)	$2.85x10^{-2}$ ($1.85x10^{-2}$ - $4.27x10^{-2}$)	$4.00x10^{-4}$ ($3.43x10^{-4}$ - $4.68x10^{-4}$)

Table 4. Cytotoxicity on tumoral cell lines of ethanol fractions from *Rollinia laurifolia*. Data are expressed as 50% inhibitory concentration (IC_{50}) . NT= no tested;. ND= no detected; E = ethanol fraction. Cell lines: Human breast carcinoma (MDA MB 231), Mouse tumorigenic fibroblast (L929), Human melanoma (Mewo) and Rat glioma (C6).

(IC_{50}=$6.43x10^{-2}$ and $3.57x10^{-2}$ µg/mL, respectively) followed by Mewo (IC_{50}=$1.07x10^{-1}$ µg/mL). Moreover, E29 was toxic on CHO cells too, (IC_{50}=3.43x 10^{-1} µg/mL) (data not shown), similar to the effect observed in Mewo cells but lower to that observed on L929 and C6 cells.

The effect of fraction E31 was strong on Mewo and L929 cell lines (IC_{50} 1.5 - $2.7x10^{-1}$ µg/mL), intermediate on MDA MB 231 (2.61 µg/mL) and nonexistent (ND) in C6 cells. The IC_{50} values for fraction E31.1 (derived from E31) was the lowest for all the cell lines IC_{50}>15 µg/mL, with the exception of C6 cells with IC_{50}=1.93 µg/mL.

E27, E29 and E31, was characterized as a complex mixture of annonaceous acetogenins by TLC analysis, IR spectra and RMN ^{1}H and was not further studied.

DISCUSSION

Annonaceous acetogenins are a class of bioactive products, associated with cytotoxic, antitumor, antimalarial, pesticidal, antifeedant, antimicrobial, pesticidal and immunossuppressive activities [3]. The annonaceous acetogenins have been isolated, so far, from the genera *Annona, Anomianthus, Asimina, Disepalum, Goniothalamus, Rollinia, Polyalthia, Porcelia, Uvaria* and *Xylopia*.

Annonaceous acetogenins are waxy substances of polyketides origin characterized by a skeleton of 35-37 carbons. Their general structures consist of a long alkyl chain bearing a terminal γ-lactone, generally 2,4-disubstituted, and a variable number of tetrahydrofuran and tetrahydropyran rings. Several oxygenated substitutions like hydroxyl, acetoxyl, ketonic, vic-diols, epoxide rings and, in some cases, a double bond have been found on the alkyl chain. Acetogenins are highly functionalized compounds presenting also several quiral centers that render their purification rather difficult. They are often obtained as a complex mixture of components, showing similar chromatographic Rf, thus posing a challenge for purification.

In this work we evaluated the cytotoxic activity of some leaf fractions from *Rollinia laurifolia,* collected at the Brazilian "cerrado", containing acetogenins identified by TLC, IR and NMR spectra. A preliminary screening of these fractions allowed determination of the LC_{50} by using the *A. salina* brine shrimp assay.

Fractions from hexanic (H) and ethanolic (E) extracts of *R. laurifolia* leaves showed $LC_{50} \leq 41.3$ μg/mL. These LC_{50} levels are considered as toxic and with potential for biologic activity, as suggested by Meyer *et al.* [12] and Rieser *et al.* [18]. The lack of positive reaction for tannins/polyphenols and saponins in most of the assayed fractions (80-90%) rule out their role as potentially cytotoxic substances.

The next step was to determine the inhibitory concentration 50% –IC_{50}– on a panel of tumor, and a normal cell line by some of the acetogenin containing fractions chosen from the *A. salina* test. According to the Rieser index 18 that sets an $IC_{50} \leq 20.0$ μg/mL for potentially bioactive substances, 67% of the H fractions and 94% of the E fractions screened deserve further analysis as potentially bioactive substances (Tables 1 and 3). Interestingly, high cytotoxicity ($IC_{50} < 10^{-1}$ μg/mL) was observed in fractions H11.12, H11.13, E13, E29 and E31. For instance, fraction H9.1 yielded gonionenin (Fig. 1-2) a C-37 mono-THF acetogenin, tetrahydroxylated in C-4, C-10, C-13 and C-18, containing one double bound in C-21, reported in this genus for the first time. This acetogenin was previously described by Gu *et al.* [16], in *Goniothalamus giganteus* bark, and reported as having cytotoxic activity on tumoral cell lines A-549, MCF-7 e HT-29.

Also, fraction H11.12 was the source of a recently described acetogenin, laurifolin (Figure 1-1). Laurifolin is a C-35 mono-tetrahydrofuran dihydroxylated acetogenin with a THF ring at C-20 [7]. Laurifolin structurally resembles the acetogenin solamin, isolated from *Annona reticulata* leaves, the difference being the positioning of the THF ring, located in C16 of laurifolin. According to Alali *et al.* [3], this substitution enhances the biological activity of acetogenins. In spite of that, the cytotoxicity of laurifolin containing fractions on tumoral cell lines is similar (IC_{50} 1.62 x 10^{-1} μg/mL – C6) when compared to solamin (IC_{50} = 3.0x10^{-1} μg/mL - 9KB cells) or lower (IC_{50} = 4.0x10^{-2} μg/mL leukemia cells - P-388) [19]. Rieser *et al.* [18] described six bioactive acetogenins in *Annona muricata* seed with structures similar to laurifolin. Their LC_{50} on *A. salina* ranged from 10^{-1} - 10^{1} μg/mL and the IC_{50}, on human carcinoma cells, was about 10^{-3} μg/mL.

Further fractionation of E13, provided annonin I (Fig. 1-3), which is a C-37 acetogenin possessing two adjacent THF rings, flanked by hydroxyl groups, and with one additional hydroxyl group in C-28. Annonin I was first reported in *Annona squamosa* and *Rollinia papilionella* seeds [6] and for the first time identified in *R. laurifolia* [9].

According to Alali *et al.* [3] acetogenins possessing two adjacent THF rings are more active than those with more distant THF-ring distribution. In this study, a single acetogenin was isolated containing an adjacent bis-THF ring that displays major potency on some of the cell lines studied ($IC_{50} \approx 10^{-2}$ μg/mL).

Considering the antitumoral potential of acetogenins, and the selective toxicity of fractions H11.12 and E29 in tumoral versus normal cells, further assessment of these fractions is required to evaluate their potential as therapeutic principles.

Acknowledgements. The authors thank CAPES, FAPEMIG and CNPq for scholarship and financial support, Angela Cristina Assunção for her assistance during the purification steps and to Prof. Dr. Antônio Jorge for mass spectra analysis.

REFERENCES

1. Correa, P. (1926) *Dicionário de Plantas Úteis e Espécies Exóticas e Cultivadas,* Imprensa Nacional, Rio de Janeiro.
2. Leboeuf, M., A. Cavé, P.K. Bhaumik, B. Mukherjee, & R. Mukherjee (1982) *Phytochem.* **21**: 2783-813.
3. Alali, F.Q., Liu, X.X., & J.L. McLaughlin (1999) *J. Nat. Prod.* **62**: 504-40.
4. Londershausen, M.W., F. Leicht, H. Lieb, T. Moeschler & H. Weiss (1991) *Pestic. Sci.* **33**: 427-38.
5. Lewis, M.A., J.T. Arnason, B.J.R. Philogene, J.K. Rupprecht, & J.L. McLaughlin (1993) *Pestic. Biochem. Physiol.* **45**: 15-23.
6. Bermejo, A., Figadère, B., Zafra-Polo, M.C., Barrachina, I., (2005) *Nat. Prod. Report* **22**: 269-303.
7. Pimenta, L.P.S., F.C. Nascimento, A.C.S. Assunção, & M.A.D. Boaventura (2001) *Tetrahedron Let.* **42**: 8433-4.
8. Nascimento, F.C., A.C.S. Assunção, M.A.D. Boaventura, & LP.S. Pimenta (2003) *Química Nova* **26**: 319-22.
9. Pimenta, Lucia P. S., Nascimento, Francisco C., Boaventura, Maria Amelia D. (2005) *Helvetica Chimica Acta* **88**: 3225-31.
10. Cavé, A., D. Cortes, B. Figadère, A. Laurens, G.R. Pettit (1997) In: Progress in the Chemistry of Organic Natural Products (W. Herz, G.W. Kirby, R.E.W. Moore, W. Steglich, C.H. Tamm, eds.). Springer-Verlag/Wien, Austria, pp. 81-288.
11. Wagner, H., S. Bladt, E.M. Zgainski (1984) Alkaloid Drugs, in *"Plant Drug Analysis A Thin Layer Chromatogaphy Atlas",* Springer-Verlag Berlin Heidelberg, pp. 51-92.
12. Meyer, B.N., N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols & J.L. McLaughlin (1982) *Planta Med.* **45**: 31-34.
13. Finney, D.J. (1976) *Probit Analysis*. Cambridge University Press, Cambridge.
14. Câmara H. (1967) *Manual de Farmacologia Prática*. São Paulo: Atheneu Editora, São Paulo S/A, pp. 40-67.
15. Bellamy, W.T. (1992) *Drugs* **44**: 690-708.
16. Gu, Z.M., X.P. Fang, L. Zeng, R. Song, J.H. Ng, K.V. Wood, D.L. Smith & J.L. Mclaughlin (1994) *J. Org. Chem.* **59**: 3472-9.
17. Rupprecht, J.K., Y.H. Hui & J.L. Mclaughlin. (1990) *J. Nat. Prod.* **53**: 237-78.
18. Rieser, M.J., Z.M. Gu, X.P. Fang, L. Zeng, K.V. Wood, & J.L. Mclaughlin (1996) *J. Nat. Prod.* **59**: 100-8.
19. Chang, F.R., Y. Wu & C. Duh Wang S.K. (1993) *J. Nat. Prod.* **56**: 1688-94.