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Bio-guided isolation of antimicrobial compounds from *Rapanea ferruginea* and its cytotoxic and genotoxic potential

Alexandre Bella Cruz[1], Katherine Kazmierczak[1], Vanessa F. Gazoni[1], Elis R. Monteiro[1], Lais M. Fronza[1], Priscila Martins[1], Rosendo A. Yunes[2], Cristiani Bürger[1], Thais A. Tomio[1], Rilton A. Freitas[1] and Angela Malheiros[1]

[1]Programa de Pós-graduação em Ciências Farmacêuticas, Universidade do Vale do Itajaí (UNIVALI), Rua Uruguai 458, 88302-202 Itajaí, SC, Brazil.
[2]Departamento de Química, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil.

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This study describes the bio-guided isolation of compounds with antimicrobial activity from *Rapanea ferruginea*, as well as the cytotoxic and genotoxic potential. The extracts of stem bark, branches, leaves, fruits and the compounds were evaluated *in vitro* against bacteria and yeast strains, and were submitted to phytochemical screening and bioautography to guide the isolation of active compounds. The toxic potential was evaluated by brine shrimp test (BST) and murine fibroblast L929 cells, and genotoxicity was evaluated using the yeast *Saccharomyces cerevisiae* (haploid wild-type). Through bioactivity-directed fractionation, the stem bark extract was chromatographed to yield the antimicrobial compounds identified as myrsinoic acids A and B (MAA, MAB) that presented significant antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*. It was verified that the compounds MAA and MAB presented moderate toxic potential on BST. However, these compounds did not present citotoxic activity on L929 fibroblasts cells and also were not mutagenic in *S. cerevisiae*. In conclusion, compounds from *R. ferruginea* might have great potential as antimicrobial agents. This study showed the antimicrobial activity of stem bark and fruits of *R. ferruginea* presented activity against Gram-positive bacteria and this effect is associated with the major percentage of MAA and MAB.

Key words: *Rapanea ferruginea*, antimicrobial, cytotoxicity, genotoxicity, medicinal plant.

INTRODUCTION

Since antiquity, man has used plants to treat common infectious diseases (Rios and Recio, 2005), and their preparations have found applications as naturally occurring antimicrobial agents in the field of pharmacology, pharmaceutical botany, phytopathology, medical and clinical microbiology, food preservation, etc. (Vukovic et al., 2007), and it is well established that natural products have been a source of leads for the development of many

*Corresponding author. E-mail: bellacruz@univali.br.

of the most effective drugs currently available for the treatment of a variety of human diseases. The exploration of nature as a source of novel active agents may serve as the leads and scaffolds for elaboration into desperately needed efficacious drugs for a multitude of disease indications (Newman and Cragg, 2012).

Plants have an exceptional ability to produce cytotoxic agents, probably because the antimicrobial natural products should be present or synthesized *de novo* in plants following microbial attack to protect the producer from pathogenic microbes in its environment (Gibbons, 2005). Over the last decade, it has become clear that antibiotics are losing their effectiveness as pathogens evolve resistance against them, a problem compounded by the fact that new drugs only rarely reach the market. Moreover, bacteria can acquire drug resistance in a multitude of ways (Saleem et al., 2010). The main advantage of natural agents is that they rarely enhance “antibiotic resistance”, a phenomenon commonly encountered with the long-term use of synthetic antibiotics (Vukovic et al., 2007).

Prenilated aromatic compounds isolated from plant sources are in the literature, including some that are diprenylated benzoic acids. This class of compounds is present in the *Rapanea* genus including myrsinoic acids A, B and C. These compounds have demonstrated anti-inflammatory activity (Hirota et al., 2002). It has been reported that myrsinoic acid A (MAA) inhibits DNA-polymerase (Mizushina et al., 2000) and methioninase inhibitor activity for myrsinoic acid B (MAB) (Ito et al., 2008). We have previously verified that MAB isolated from the bark of $CHCl_3$ extract of *Rapanea ferruginea,* synonym *Myrsine coriacea* (Myrsinaceae), exhibits significant and potent antinociceptive action when evaluated in some pharmacological models of pain in mice (Hess et al., 2010).

In Brazil, *R. ferruginea* (synonym *Myrsine floculosa* Mar., *M. coriacea* (Ruiz and Pavon) Mez and *Gaballeria ferruginea* (Ruiz and Pavon) (Myrsinaceae), it is popularly known as "canela-azeitona", "capororoca", "azeitona-do-mato", "camará", "capororocaçu", "capororoca-vermelha", "pororoca" and "capororoca-mirim". This plant is used to feed wildlife, and has also been used as a condiment, charcoal production, construction, firewood, landscaping, reforestation and environmental restoration (Pascotto, 2007). The tea leaves or the bark of *R. ferruginea* is indicated as a diuretic, to combat diseases of the urinary tract and also is a good cleanser. It is popularly used for itching, rashes, hives, eczema, rheumatism and diseases of the liver (Lorenzi, 1992).

In order to detect antimicrobial compounds in extracts of plants, we report here the antimicrobial activity, as well as the cytotoxicity and genotoxicity activity of extracts and myrsinoic acids MAA and MAB from *R. ferruginea*.

MATERIALS AND METHODS

Plant

Rapanea ferruginea, synonym *M. coriacea* (Ruiz and Pavon) Mez (Myrsinaceae) was collected at Blumenau (Santa Catarina, Brazil) in July, 2007 and identified by Oscar Benigno Iza (Universidade do Vale do Itajaí). A voucher for this plant was deposited at the Herbarium Barbosa Rodrigues, in Itajaí, under number HBR 52715.

Extraction and isolation procedures

Stem bark of *R. ferruginea* (600 g) was dried at 40°C for seven days, powdered and successively extracted by maceration with $CHCl_3$ for seven days at room temperature. The extract was concentrated under reduced pressure to yield residues (70.2 g). Part of the $CHCl_3$ extract (63.4 g) was subjected to column chromatography (Φi 9 cm) packed with silica gel 60 to 230 mesh and eluted with hexane gradually enriched in ethyl acetate. The fraction was eluted with 20% ethyl-acetate in hexane to provide MAA 462 mg. The fraction was eluted with 40% ethyl-acetate in hexane to provide impure MAB. This fraction (8.5 g) was rechromatographed in a silica-gel column with hexane/ethyl acetate to provide pure MAB (4.3 g). The myrsinoic acids were characterized by nuclear magnetic resonance.

Spectral data MAA

^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.60 (3H, s, H-10’), 1.69 (3H, s, H-8’), 1.77 (9H, s, H-4”, 5”, 9’), 2.11 (2H, m, H-5’), 3.38 (2H, t, *J* = 6.0 Hz), 3.39 (2H, t, *J* = 6.0 Hz), 5.08 (1H, m, H-6’), 5.32 (2H, t, *J* = 7.0 Hz, 2H’, 2”), 7.77 (2H, s, H-2, H-6). ^{13}C-NMR ($CDCl_3$, 75,5 MHz) δ: 16.2 (C-9’), 17.9 (C-10’, 4”), 25.7 (C-8’), 25.8 (C-5”), 26.3 (C-5’), 29.3 (C-1”), 29.7 (C-1’), 39.7 (C-4’), 121.0 (C-1), 121.2 (C-2’), 121.4 (C-2”), 123.8 (C-6’), 127.0 (C-3, C-5), 130.5 (C-2, C-6), 131.8 (C-7’), 134.0 (C-3”), 139.1 (C-3’), 158.0 (C-4), 172.0 COOH.

Spectral data MAB

^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.30 (3H, s, H-7’), 1.55 (2H, m, H-2’), 1.63 (3H, s, H-8’), 1.69 (3H, s, H-6’),1.72 (3H, s, H-5”), 1.73 (3H, s, H-4”), 2.17 (2H, m, H-3’), 3.17 (2H, dd, *J* =16,0, 9,2 Hz, H-3), 3.29 (2H, m, H-1”), 4.74 (1H, t, *J* = 9.2 Hz, H-2), 5.12 (1H, t, *J* = 7.2 Hz, H-4’), 5.28 (1H, t, *J* = 7.2 Hz, H2”), 7.75 (2H, s, H-4, H-6). ^{13}C-NMR ($CDCl_3$, 75.5 MHz) δ: 17.7 (C-8’), 17.9 (C-5”), 22.0 (C-3’), 22.6 (C-7’), 25.6 (C-4”), 25.7 (C-6’), 28.3 (C-1”), 29.9 (C-3), 37.1 (C-2’), 73.8 (C-1’), 89.6 (C-2), 121.4 (C-2”), 121.8 (C-5), 123.1 (C-3a), 124.0 (C-4’), 125.0 (C-6), 127.2 (C-7), 131.4 (C-4), 132.2 (C-5’), 133.2 (C-3”), 162.4 (C-7a), 171.7 (C8).

To evaluate the profile of the different parts of the plant, leaves (113 g), branches (843 g) and fruits (276 g) were dried in oven with air circulation at 35°C for seven days, powdered and sieved. Alcoholic extracts were prepared by maceration with 96% ethanol for 7 days, and then filtered. The extracts were concentrated under reduced pressure and kept in an amber flask (leaves 7.54 g, branches 8.48 g, fruits 8.36 g).

Microorganisms

For the antimicrobial evaluation, strains were used from the American Type Culture Collection (ATCC), Rockville, MD, USA,

Bacteria: *Bacillus subtilis* ATCC 23858, *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 25933, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus saprophyticus* ATCC 35552, *Streptococcus pyogenes* ATCC 19615, and yeast *Candida albicans* ATCC 10231, *Candida krusei* ATCC 6582, *Candida parapsilosis* ATCC 22019, *Saccharomyces cerevisiae* ATCC 9763. For mutagenic effects, the *S. cerevisiae* (haploid wild-type) XV185-14c (MATα, *ade*2-2, *arg*4-17, *his*1-7, *lys*1-1, *trp*5-48, *hom*3-10) was provided by Dr. R. C. Von Borstel (Genetics Department, Alberta University, Canada).

Media and innocula

The bacteria used were cultivated in Mueller-Hinton agar (MHA - Difco) at 35°C for 24 h. Cell suspension in saline (0.86%) was adjusted to give a final concentration of 1.5×10^8 cell ml^{-1}, standardized with 0.5 on the McFarland scale (λ = 530 nm) (Clinical and Laboratory Standards Institute (CLSI), 2009). The yeast were cultivated in Sabouraud dextrose agar (SDA - Difco) and prepared according to Pfaller et al. (1988), adjusting the suspension to give a final concentration between 1.0×10^6 and 5.0×10^6 cell ml^{-1}, which was also standardized with 0.5 on the McFarland scale (λ = 530 nm).

Thin layer chromatography

Silica gel 60 F_{254} aluminium sheets, 0.2 mm thick (Merck), 10 × 10 cm, were used, plant extracts (8 μg $μl^{-1}$) and pure isolated compounds MAA and MAB (2 μg $μl^{-1}$) were applied (6 μl) and the chromatogram was developed using hexane:ethyl acetate (60:40) as eluent. Thin layered chromatography (TLC) plates were run and spots and bands were visualized by ultra violet (UV) irradiation (254 nm).

Bioautography

Chromatograms developed as described above were placed in a square plate with a cover, and an inoculum of *S. aureus* containing 10^6 colony-forming units (CFU) ml^{-1} in molten MHA was distributed over the plate. After solidification of the medium, the TLC plate was incubated overnight at 35°C. Subsequently, the bio-autogram was sprayed with an aqueous solution of 2,3,5-triphenyltetrazolium chloride and incubated at 35°C for 4 h. Inhibition zones indicated the presence of active compounds.

Quantitative antimicrobial evaluation

The minimum inhibitory concentration (MIC) was determined by the agar dilution method which was carried out in slants (1 ml). Stock solutions of each extract and compound in dimethylsulfoxide (DMSO) was diluted to provide serial two-fold dilutions which were added to each medium (MHA for bacteria, and SDA for yeast), resulting in concentrations ranging from 1 to 1000 μg ml^{-1}. Afterward, a volume of 1 μl of inoculum suspension, prepared previously, was added to each slant, with the exception of the sterile control. The slants were incubated at 35°C. Only *S. pyogenes* growing in MHA with 5% sheep blood incubated in a CO_2-enriched atmosphere. The MICs were visually recorded after 24 h for the bacteria and 48 h for the yeasts. The final concentration of DMSO in the assay did not exceed 2%, and the antibacterial agent ampicillin (Sigma-Aldrich) was included in the assay as positive control, and drug-free solution was also used as a black control. Each assay was repeated three times. The MICs were determined as the lowest concentration of extract or compound inhibiting visible growth of each organism in the agar slant.

Brine shrimp test (BST)

Dried *Artemia salina* eggs (1 g L^{-1}) (Maramar, Arraial do Cabo, RJ, Brazil) were placed in artificial sea water according Lewan et al. (1992) and incubated at 22 to 29°C with strong aeration, under a continuous light regime (2000 Lux). Approximately 12 h after hatching, the phototropic nauplii were collected for the cytotoxicity assay. Serial dilutions were made in the wells of 96-well microplates in triplicate in 100 μl artificial sea water. Control wells with DMSO were included in each experiment. The solvents and reagents employed on study were endotoxin free. A suspension of nauplii containing ten organisms was added to each well and the covered plate were incubated at 22 to 29°C. The toxicity was determined after 24 h of exposure. The number of survivors was counted. Larvae were considered dead if they did not exhibit any internal or external movement during several seconds of observation (Solis et al., 1993), and LC_{50} values were then calculated by Probit analysis (Finney, 1971).

Cytotoxicity assay (murine fibroblast L929)

The L929 non-tumor cell line (a murine fibroblast-like cell) was originally obtained from the Rio de Janeiro Cell Collection (Banco de Células do Rio de Janeiro - BCRJ, Brazil). Cells were maintained at 37°C in a humidified atmosphere (90%) containing 5% CO_2 and subcultured every 3 to 4 days. The cells were cultured in Eagle's minimum essential medium with 10% of fetal bovine serum (MEM + FBS), with 1% PSN antibiotics (5 mg ml^{-1} penicillin, 5 mg ml^{-1} streptomycin, 10 mg ml^{-1} neomycin), 0.5 antifungal (250 μg ml^{-1} amphotericin), 2 mM L-glutamine and 2.2 mg ml^{-1} bicarbonate sodium at 37°C in a humidified atmosphere containing 5% CO_2. The cell viability was determined using a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) assay (Mosmann, 1983). Briefly, cells were seeded (10^4 cells $well^{-1}$) onto flat-bottomed 96-well culture plates. Extracts and compounds were diluted to achieve different concentrations (100 to 0.2 μg ml^{-1}) in the wells and allowed to grow for 48 h. For each concentration, there was a control sample that remained untreated and received an equal volume of medium. After removing the medium, cells were then labeled with MTT solution (5 mg ml^{-1} in PBS) for 4 h and the resulting formazan was solubilized with DMSO (100 μL). Absorbance was measured at 570 nm using an microplate reader. Cell viability was expressed as a percentage of the control culture value. Experiments for each extract and compound were carried out in triplicate, including untreated cell control and a blank cell-free control. The cytotoxic effects of the *Rapanea* extracts and compounds was expressed as the IC_{50} value (the drug concentration reducing the absorbance of treated cells by 50% with respect to untreated cells). The data were expressed as mean ± standard deviation. Statistical analysis was performed using GraphPad Instat software, version 3.05. The values were analyzed using one-way analysis of variance (ANOVA), followed by a Tukey test where appropriate p values ($p < 0.05$) were considered indicative of significance.

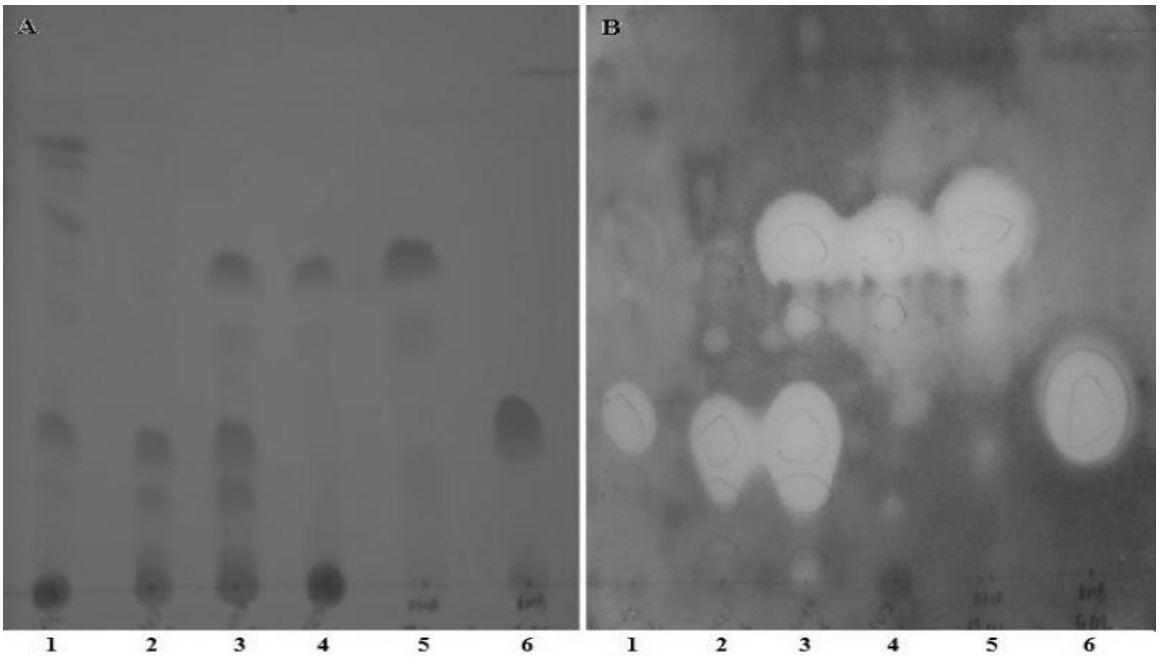

Figure 1. Thin layer chromatography plate and bioautography of extracts and myrsinoic acids A and B isolated from *R. ferruginea*.
(A) visualized by UV irradiation (254 nm), (B) bioautography with *Staphylococcus aureus.* (1) alcoholic extract from leaves; (2) alcoholic extract from branches; (3) chloroform extract from stem bark; (4) ethanolic extract from fruits; (5) myrsinoic acid A; (6) myrsinoic acid B.

Mutagenicity effects on *S. cerevisiae*

Mutagenisis was measured in haploid cells of stationary phase culture of strain XV185-14c, as described in Costa et al. (2008). A suspension of 2×10^8 cells ml^{-1} of the yeast strain XV185-14c grew until the exponential phase was incubated for 1 h at 800 rpm in several different concentrations (25, 62.5, 125, 250 and 500 µg ml^{-1}) of myrsinoic acids (MAA and MAB). Survival was determined based on *S. cerevisiae* (SC) (5 days, 30°C), and mutation induction (*lys*+, *his*+ or *hom*+ revertants) was performed in appropriate deficient media. While *his* 1-7 is a non-suppressible missense allele, and reversions result from mutations at the locus 24 itself, *lys* 1-1 is a suppressible ochre nonsense mutant allele which can be reverted either by locus-specific or by forward mutation in a suppressor gene, and it is believed that *hom* 3-10 contains a frame shift mutation due to its response to a range of diagnostic mutagens 25. Assays were repeated at least twice, with plating done in triplicate for each dose. 4-nitroquinolein-1-oxide (4-NQO, 5 µM) was used as mutagenic positive control. The solvents and reagents employed on study were endotoxin free. The data were expressed as mean ± standard deviation. Statistical analysis was performed using GraphPad Instat software, version 3.05. The values were analyzed using one-way analysis of variance (ANOVA), followed by a Dunnett multiple comparison test where appropriate p values ($p < 0.05$, $p < 0.01$ and $p < 0.001$) were considered indicative of significance.

RESULTS AND DISCUSSION

The bioautographic assay is a preliminary study to verify the possibility of antimicrobial activity in plant extracts. The extracts obtained from different parts of *R. feruginea* and compounds previously isolated from stem barks, MAA and MAB, were available in TLC plates. Figure 1A shows the TLC visualized in UV λ = 254 nm. In fruits and stem bark extracts, a spot with *Rf* 0.64 was observed occurring with MAA. In branch, leaf and stem bark extracts, another spot with *Rf* = 0.30 is evident, occurring with MAB. In these extracts, a spot with *Rf* = 0.18 was observed, and in stem bark and fruit extracts another zone with *Rf* = 0.52 was observed, but these compounds were not identified.

TLC plate was assayed for bioautography (Figure 1B) indicating the location of a bacterial inhibition zone against *S. aureus*. MAA and MAB were the principles responsible for the activity observed. Other compounds also presented an inhibition zone (*Rf* = 0.52 and 0.18). In this experiment, the predominance of MAA in fruit extracts can be observed. In stem bark extracts, MAA and MAB can be observed. In branch and leaf extracts, the presence of MAB can be observed, but their inhibition zones had a lower intensity compared with stem bark extracts. This experiment shows that MAA and MAB are principally responsible for activity in this species.

Antimicrobial activity of the extracts and compounds of *R. ferruginea* against bacteria and yeast were determined

Table 1. Antimicrobial activity of *Rapanea ferruginea* extract and compounds.

Component	Minimal inhibitory concentration (µg ml^{-1})					
	B. subtilitis	***S. aureus***	***S. saprophyticus***	***S. pyogenes***	**Gram-negative**	**Yeast**
Rf stem bark	125	62.5	62.5	500	>1000	>1000
Rf fruit	125	31.25	500	500	>1000	>1000
Rf leaves	500	500	250	>1000	>1000	>1000
Rf branch	250	500	250	500	>1000	>1000
MAA	7.81	31.25	31.25	125	>1000	>1000
MAB	62.5	31.25	125	125	>1000	>1000
Ampicillin	12.5	0.12	0.12	0.25	7.0$^{\#}$	nt

B. sub = Bacillus subtilis), *S. aur = Staphylococcus aureus*), *S. sap* (*Staphylococcus saprophyticus*), *S. pyo* (*Streptococcus pyogenes*), Gram-neg (Gram-negative bacteria), $^{\#}$(result against *Escherichia coli*), nt (not tested), Rf stem bark (chloroform extract from stem bark of *Rapanea ferruginea*), Rf fruit (ethanolic extract from fruit), Rf leaves (ethanolic extract from leaves), Rf branch (ethanolic extract from branch), MAA (myrsinoic acid A), MAB (myrsinoic acid B).

by the dilution agar assay because this method is especially good for determining the relative potency of polar and non-polar substances as well as all types of complex extracts (Rios and Recio, 2005). The results of minimal inhibitory concentrations are shown in Table 1 and are classified as described in Holetz et al. (2002).

Fruit and stem bark extracts presented higher activity against the same bacteria, with MICs between 31.25 to 500 µg ml^{-1}. As demonstrated in the bioautography, MAA and MAB are the main compounds in these extracts and are probably responsible for observed activity. MAA is present in the fruit extract and presented a good prominent activity against Gram-positive bacteria with MIC ranging from 7.81 to 125 µg ml^{-1}. MAB is predominant in stem bark with MIC from 31.5 to 125 µg ml^{-1}. The main difference were observed against *B. subtilis* and *S. saprophyticus*, where MAA exhibited MIC between 7.81 and 31.25 µg mL^{-1} and MAB exhibited MIC between 62.5 and 125 µg ml^{-1}, respectively. The better results to MAA can be explained with the presence of phenol in it structure. It is known that phenols are predominantly membrane-active agents, especially *p*-substituted phenols. This class of compounds damage cell membrane and cause release of intracellular constituents. They also cause intracellular coagulation of cytoplasm constituents leading to cell death or inhibition of cell growth (Russell and Chopra, 1996).

Leaf and branch extracts displayed moderate or weak activity against Gram-positive bacteria. In contrast to the relative low MICs for Gram-positive bacteria to fruits, stem bark extracts, MAA and MAB, Gram-negative bacteria and yeast were not inhibited by extracts and compounds up to 1,000 µg ml^{-1}. One helpful clue regarding the possible function of plant secondary metabolites is that these compounds often show considerable activity against Gram-positive bacteria but not against Gram-negative species or yeast (Lewis, 2001). Both yeast and Gram-negative bacteria have evolved significant permeability barriers (Tegos et al., 2002).

Initially, BST was used to obtain some idea of their possible effects since this assay has been considered a useful tool for preliminary assessment of toxicity potential (Meyer et al., 1982; Colegate and Molyneux, 2008). According to Dolabela (1997), an LC_{50} < 80 µg ml^{-1} is considered very toxic, LC_{50} 80 to 250 µg mL^{-1} considered moderate, and LC_{50} > 250 µg mL^{-1} considered as weak or nontoxic. Regarding the results of the BST (Table 2), the extracts showed weak toxicity (LC_{50} = 262.45 to 462.17 µg ml^{-1}); therefore, the compounds MAA and MAB presented moderate toxic potential with LC_{50} value of 91.65 and 182.39 µg ml^{-1}, respectively.

Subsequently, the cytotoxicity was also evaluated using L929 cell and the results indicated that only the extract of the bark showed toxic effect at a concentration of IC_{50} = 56.04 µg ml^{-1}. However, for the other extracts and the compounds MAA and MAB, there was no observed reduction in cell viability. Comparing the results between the BST and L929 assays, it was verified that there are some discrepancy. The differences between the metabolic pathways of organisms as brine shrimp and those present in more complex cells (mammal) can justify the discrepancies in the results. Although the brine shrimp lethality assay (BST) is considered a useful tool for preliminary assessment of toxicity (Solis et al., 1993), as well as a wide variety of biologically active chemical compounds. In particular, cytotoxic agents are toxic to brine shrimp (Déciga-Campos et al., 2007), others studies found no correlation between *Artemia salina* mortality and mammal cell lines inhibition values (Berry et al., 2004; Hisem et al., 2011). On the other hand, the cytotoxic effect also was evaluated concomitantly with genotoxicity assay of MAA and MAB in the haploid strain of *S. cerevisiae* (XV185-14c), and was verified equivalent to IC_{50} = 66.64 and 279.92 µg ml^{-1}, respectively, reducing the

Table 2. Cytotoxicity activity of extracts and compounds of *R. ferruginea* in BST, murine fibroblast and yeast cells.

Component	Cytotoxicity activity (µg ml^{-1})		
	BST (LC_{50})	L929 (IC_{50})	Yeast (IC_{50})
Rf stem bark	376.16	56.04	n.t.
Rf fruit	262.45	>100	n.t.
Rf leaves	462.17	>100	n.t.
Rf branch	420.67	>100	n.t.
MAA	91.65	>100	66.64
MAB	182.39	>100	279.92

BST (brine shrimp test), L929 (murine fibroblast L929 cells), Yeast (*Saccharomyces cerevisiae* strain XV185-14c), LC_{50} (lethal concentration 50%), IC_{50} (inhibitory concentration 50%), n.t. (not tested), Rf (*Rapanea ferruginea*), MAA (myrsinoic acid A), MAB (myrsinoic acid B).

cell viability compared to the negative control (Table 2), corroborating at least in part, with the results obtained with BST.

With regard to the genotoxic potential, it was verified that the compounds did not induce mutations to lysine and histidine (a locus-specific mutation) or homoserine (frameshift events), showing no statistical differences between both compounds with the negative control. Although the results of initial tests for *in vitro* cytotoxicity assays present limitations as to its direct correlation with clinical situations and cannot be extrapolated to humans, they are very important because they determine the biological behavior of the material submitted for evaluation, representing an important step before clinical trials.

Conclusion

This study showed that the antimicrobial activity of stem bark and fruits of *R. ferruginea* presented selective antimicrobial activity against Gram-positive bacteria (*B. subtilis, S. aureus, S. saprophyticus* and *S. pyogenes*). This effect is associated with the major percentage of myrsinoic acids A and B, and it is the first study to demonstrate the antimicrobial activity. Also, these compounds presented moderate toxicity in BST, but no presented cytotoxic activity on L929 fibroblasts cells and also no mutagenic effect was detected for *S. cerevisiae* (XV185-14c).

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