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Original Article

Antimicrobial activity of the myrsinoic acid A from *Myrsine coriacea* and the semi-synthetic derivatives

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ABSTRACT

The antimicrobial activity of the myrsinoic acid A isolated from *Myrsine coriacea* (Sw.) R.Br. ex Roem. & Schult., Primulaceae, and a two semi-synthetics derivatives was tested against *Bacillus subtilis*, *Escherichia coli*, *Salmonella enterica* subsp. *enterica serovar typhi*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Candida albicans*, *Candida krusei* and *Candida tropicalis*. The microdilution method was used for the determination of the minimum inhibitory concentration during evaluation of the antimicrobial activity. The myrsinoic acid A showed no activity against the selected microorganisms but the hydrogenated and acetylated derivatives were active against *B. subtilis*, *E. coli*, *S. aureus* and *P. aeruginosa*.

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Introduction

Bacterial infectious diseases are a serious worldwide public health problem. The increase in bacterial resistance and the rapid emergence of new infections have drastically decreased the efficacy of the drugs employed in the treatment of pathologies caused by certain microorganisms (Hemaiswarya et al., 2008). The problem of microbial resistance is growing and the future prospect of the use of antimicrobial drugs is uncertain. It has become urgent to adopt, therefore, measures to tackle the problem, including the control of the use of antibiotics and studies on new natural and synthetic drugs (Nascimento et al., 2000).

The plant *Myrsine coriacea* (Sw.) R.Br. ex Roem. & Schult. is owned by the family Primulaceae and gender *Myrsine*, characterized by alquilbenziquinonas and triterpenoids, the plants of this genus are known as capororoca, and several studies have reported biological activities such as cytotoxic, contraceptive, biocidal, anti-spermatogenic, antiseptic, antibacterial, anti-inflammatory, antioxidant, antiplasmodial, and against diabetes mellitus (Bagalwa and Chifundera, 2007; Bhandari et al., 2008; Cordero et al., 2004; Gathuama et al., 2004; Januário et al., 1991, 1992; Katuura et al., 2007; Reguero et al., 1989).

Several natural products of plants have been used as basic structures for the synthesis and development of new drugs, so the natural products have been used as starting to select lead compounds for optimization.

This study was conducted to determine the *in vitro* antimicrobial activity of myrsinoic acid A from the plant *M. coriacea*, and their derivatives.

Materials and methods

Plant material

Myrsine coriacea (Sw.) R.Br. ex Roem. & Schult., Primulaceae, was collected at the Cerrado of the Universidade Estadual de Goiás, Anápolis, Goiás, Brazil and identified by Dra Maria de Fatima Freitas, UFRJ, Brazil and previous as *Myrsine cuneifolia* by Dr. Mirley Luciene dos Santos, UEG, Brazil (Burger et al., 2012). Voucher specimens (4215) was deposited in the Herbarium of the same University.

Extraction, isolation and reactions

In previous work, Burger et al. (2012) isolated myrsinoic acid A (**1**) from fruits ethanolic extract of the *M. coriacea* and this

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compound was submitted to hydrogenation reaction yielding the compound **2**. The compound **3** was obtained by acetylation reaction of compound **1**. The purity of compounds **1**, **2** and **3** was determined by TLC and analyzed of their ^{1}H NMR spectrum and no contamination and by-products was detected.

Hydrogenation reaction conditions

Myrsinoic A acid (**1**) (0.02 g) was solubilized in 4 ml of methanol, and added 0.01 g of palladium–carbon (Pd–C), and allowed to contact with hydrogen gas for 12 h under constant agitation, after this time the solution was filtered with celite, the solvent was evaporated and was obtained 0.017 g (85%) of **2**.

Acetylation reaction conditions

Myrsinoic A acid (**1**) (0.02 g) was solubilized in 1 ml of pyridine and added 1 ml of acetic anhydride, and the reaction was agitated during 24 h. After, 3 ml of distillated water was added to the solution and was extracted with chloroform (5 ml) and this extract was washed with HCl (10%, 3 ml), followed by drying with magnesium sulfate, yielding 0.021 g (95%) of **3**.

Antimicrobial assay

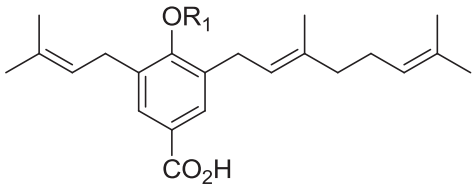
Antimicrobial assays were performed using the broth microdilution technique proposed by the Clinical and Laboratory Standards Institute (CLSI) 21 M7-A6 and adaptation of the M27-A2, for determining the MIC (lowest concentration able to inhibit the growth of microorganisms). The MIC determinations were performed in triplicate in microplates with 96 wells. The microorganisms used were from American Type Culture Collection of *Bacillus subtilis* (ATCC 6623), *Escherichia coli* (ATCC 25922), *Salmonella enterica* subsp. *enterica serovar typhi* ATCC 6539, *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* ATCC 19615, *Pseudomonas aeruginosa* (ATCC 15442), *Micrococcus luteus* ATCC 9341, *Candida albicans* (ATCC 10231), *Candida krusei* ATCC 6258, and *Candida tropicalis* ATCC 28707. The stock solutions of tested samples were prepared in vial like Eppendorf solubilizing 1 mg of sample in 40 μl of dimethyl sulfoxide (DMSO). These solutions were diluted in 960 μl of Mueller–Hinton broth for bacteria or Sabouraud broth for testing with yeast. Solutions with the final concentrations ranging from 7.81 to 500 μg/ml were prepared. The inoculums were standardized based on a scale of 0.5 McFarland turbidity standard (10^{8} CFU/ml) and diluted at 1:10 ratio to the broth microdilution procedure. After micropipetting, microplates were capped and incubated at 37 °C for 18–24 h without agitation. After the incubation period, the results were visualized and the wells that showed no apparent growth were selected to determine the antimicrobial activity of samples.

This determination was performed using subcultures in Petri dishes using Mueller–Hinton agar for growing bacteria and Sabouraud agar for the fungus growth. The Petri dishes were incubated at 37 °C for 48 h and verified the presence/absence of microbial colonies. After preparation of subcultures, were added to each well of the microplates, 15 μl of resazurin to 0.01% in sterile aqueous solution which, after 4 h of reincubation, the reading was performed. Thus, it was possible to determine the lowest concentration of each extract that can inhibit the growth of microorganisms through indicators in diluted solution.

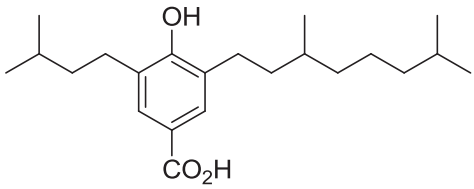
All tests were performed with negative control (DMSO), growth control of the microorganisms and control of precipitation of the sample, avoiding possible false-negative or false-positive.

Results and discussion

The compound **1** (myrsinoic A acid) from *M. coriacea* fruits ethanolic extract was submitted a catalytic hydrogenation and acetylation reactions to obtain two semi-synthetic derivatives **2** and **3**. Myrsinoic acid A (**1**) and the derivatives **2** and **3** were identified through ^{1}H and ^{13}C NMR, experiments in one dimensions (400 MHz, $CDCl_3$, Burger et al., 2012) in comparison with literature (Table 1, Cruz et al., 2013; Dong et al., 1999).



1 R_1=H
3 R_1=$COCH_3$



2

Compounds **1**–**3** were assayed in inhibition of *B. subtilis*, *E. coli*, *S. enterica* subsp. *enterica serovar typhi*, *S. aureus*, *S. pyogenes*, *P. aeruginosa*, *M. luteus*, *C. albicans*, *C. krusei* and *C. tropicalis* and reported here, by the first time, the effect of these compounds on the microorganisms tested.

The myrsinoic acid A showed no activity against the selected microorganisms but the derivatives, **2** and mainly **3**, were active against *B. subtilis*, *E. coli*, *S. aureus* and *P. aeruginosa*, results including the positive controls are described in Table 2. Cruz et al. (2013) had assayed myrsinoic acid A and it presented minimal inhibitory concentration of 7.81, 31.25, 125, >1000 and >1000 μg/ml to *B. subtilis* (ATTC 23858), *S. aureus* (ATCC 6538P), *Staphylococcus saprophyticus* (ATCC 35552), *S. pyogenes* (ATCC 27853), Gram-negative and Yeast, respectively and cytotoxicity activity to brine shrimp test (BST, LC_{50} 91.65 μg/ml), murine fibroblast L929 cell, (L929, IC_{50} >100 μg/ml) and *Saccharomyces cerevisiae* strain XV185-14c (IC_{50} 66.64 μg/ml). Myrsionic acid A also presents inhibitions to HB-EGF (heparin-binding epidermal growth factor-like growth factor; involved in cancer disease; Lee and Mandinova, 2011), to methioninase (L-methionine lyase; involved in cancer disease and oral malodor; Itoh et al., 2014), to acetylcholinesterase (related to Alzheimer disease; Gazoni, 2009; Filippin et al., 2009), and anti-inflammatory activity (Dong et al., 1999).

The results suggest that the structural changes are important for the potential inhibition against the microorganisms *B. subtilis*, *E. coli*, *S. aureus* and *P. aeruginosa*. It was noticed that the double bonds, as well as the phenolic group are not required for the activity in the microorganisms tested. Nevertheless, the ester group of the compound **3** is important for the inhibition and also showed good activity. These results can encourage more studies using this kind of compound for modifications aiming antimicrobial activity.

Table 1
Data of NMR of **1**–**3** ($CDCl_3$, 400 or 300[a] or 500[b] MHz, ^1H and 100 or 75[a] or 125[b] MHz ^{13}C, δ or ppm, J in Hz, * changeable).

^1H NMR	**1**	**1**[a]	**1**[b]	**2**	**3**	**3**[b]	^{13}CNMR	**1**	**1**[a]	**1**[b]	**3**[b]
1							1	120.9	121.0	123.8	125.9
2	7.68, s	7.77, s	7.77, s	7.76, s	7.83, s	7.84, s	2	130.0	130.5	130.5	134.5
3							3	127.0*	127.0	127.4*	127.0*
4							4	157.4	158.0	158.0	151.9
5							5	126.6*	127.0	127.0*	129.8*
6	7.68, s	7.77, s	7.77, s	7.76, s	7.83, s	7.83, s	6	130.0	130.5	130.5	134.5
1′	3.33, d, J = 7.2	3.38, t = 7.0	3.38, t = 7.0	2.62, m	3.23, d, J = 7.2*	3.24, d, J = 6.3	1′	29.0*	29.7	29.7*	28.9*
2′	5.25, brt, J = 7.2	5.32, t = 7.0	5.32, t = 6.8	1.10–1.68, m	5.25, brt, J = 7.2	5.25, brt, J = 7.1*	2′	120.8*	121.2	121.4*	120.9*
3′				1.10–1.68, m			3′	138.6	139.1	139.1	137.6
4′	2.04, m	2.11, m	2.09, m	1.10–1.68, m	2.08, m	2.10, m	4′	39.4	39.7	39.7	39.7
5′	2.04, m	2.11, m	2.14, m	1.10–1.68, m	2.08, m	2.10, m	5′	26.0	26.3	26.4	26.6
6′	5.01, brt, J = 7.2	5.08, m	5.08, brt, J = 6.4	1.10–1.68, m	5.11, brt, J = 7.2	5.11, brt, J = 6.7	6′	123.5	123.8	123.8	124.1
7′				1.10–1.68, m			7′	134.5	131.8	132.0	131.6
8′	1.61, s	1.69, s	1.68, s	0.87, d, J = 6.6	1.60, s	1.60, s	8′	25.3	25.7	25.7	25.7
9′	1.71, s	1.77, s	1.77, s	0.97, d, J = 6.6	1.70, s	1.69, s	9′	17.3	16.2	16.2	16.6
10′	1.53, s	1.60, s	1.60, s	0.96, d, J = 6.6	1.69, s	1.67, s	10′	15.9	17.9	17.7	17.7
1″	3.33, d, J = 7.2	3.38, t = 7.0	3.38, t = 7.0	2.62, m	3.24, d, J = 7.2*	3.24, d, J = 6.3	1″	29.3*	29.3	29.4*	28.8*
2″	5.25, brt, J = 7.2	5.32, t = 7.0	5.32, t = 6.8	1.10–1.68, m	5.25, brt, J = 7.2	5.24, brt, J = 7.2*	2″	121.0*	121.4	121.2*	120.8*
3″				1.10–1.68, m			3″	132.6	134.0	134.9	133.9
4″	1.71, s*	1.77, s	1.77, s	0.87, d, J = 6.6	1.70, s	1.70, s	4″	25.4	17.9	25.8	25.7
5″	1.70, s*	1.77, s	1.77, s	0.97, d, J = 6.6	1.76, s	1.76, s	5″	17.5	25.8	17.9	17.9
							COO	170.4	172.0	172.2	170.7
CH_3CO					2.33, s	2.33, s	CH_3CO				20.5
							CH_3CO				168.5

[a] Cruz et al. (2013).
[b] Dong et al. (1999).

Table 2
Activity against the microorganisms by myrsinoic A acid and derivatives.

Compounds	Minimum inhibitory concentration (μg/ml)									
	Bs	*Ec*	*St*	*Sa*	*Sp*	*Pa*	*Ml*	*Ca*	*Ck*	*Ct*
1	–	–	–	–	–	–	–	–		–
2	15.6	15.6	–	15.6	–	15.6	–	–	–	–
3	62.5	125	–	125	–	125	–	–	–	–
Vancomycin	0.047									
Chloridrate of tetracycline		3.13	0.78	0.75	0.094	1.56	0.19			
Nystatin								25.0	25.0	50.0

Positive controls: Vancomycin; Chloridrate of tetracycline; Nystatin; *Bs*, *Bacillus subtilis*; *Ec*, *Escherichia coli*; *St*, *Salmonella enterica* subsp. *enterica serovar typhi*; *Sa*, *Staphylococcus aureus*; *Sp*, *Streptococcus pyogenes*; *Pa*, *Pseudomonas aeruginosa*; *Ml*, *Micrococcus luteus*; *Ca*, *Candida albicans*; *Ck*, *Candida krusei*; *Ct*, *Candida tropicalis*; **–**, no inhibition.

Authors' contribution

MCMB and GSOC (PhD students) contributed in collecting plant sample, running the laboratory work, in the synthesis of derivatives, analysis of the data and drafted the paper. APT contributed to biological studies, analysis of the data and drafted the paper. JBF, MFGFS, PCV, ACSM contributed to chromatographic analysis, designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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