Antibacterial Activity of Triterpene Acids and Semi-Synthetic Derivatives against Oral Pathogens

Luis C. Scalon Cunha, Márcio L. Andrade e Silva, Niege A. J. Cardoso Furtado, Adriana H. C. Vinhólis, Carlos H. Gomes Martins, Ademar A. da Silva Filho, and Wilson R. Cunha*

Universidade de Franca, Av. Dr. Armando Salles de Oliveira, 201 Parque Universitário, Franca-SP, CEP 14404-600, Brazil. Fax: ++55-16-3711 8878. E-mail: wrcunha@unifran.br

* Author for correspondence and reprint requests

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Triterpene acids (ursolic, oleanoic, gypsogenic, and sumaresinolic acids) isolated from *Miconia* species, along with a mixture of ursolic and oleanolic acids and a mixture of maslinic and 2-α-hydroxyursolic acids, as well as ursolic acid derivatives were evaluated against the following microorganisms: *Streptococcus mutans*, *Streptococcus mitis*, *Streptococcus sanguinis*, *Streptococcus salivarius*, *Streptococcus sobrinus*, and *Enterococcus faecalis*, which are potentially responsible for the formation of dental caries in humans. The microdilution method was used for the determination of the minimum inhibitory concentration (MIC) during the evaluation of the antibacterial activity. All the isolated compounds, mixtures, and semi-synthetic derivatives displayed activity against all the tested bacteria, showing that they are promising antiplaque and anticaries agents. Ursolic and oleanolic acids displayed the most intense antibacterial effect, with MIC values ranging from 30 μg/mL to 80 μg/mL. The MIC values of ursolic acid derivatives, as well as those obtained for the mixture of ursolic and oleanolic acids showed that these compounds do not have higher antibacterial activity when compared with the activity observed with either ursolic acid or oleanolic acid alone. With regard to the structure-activity relationship of triterpene acids and derivatives, it is suggested that both hydroxy and carboxy groups present in the triterpenes are important for their antibacterial activity against oral pathogens.

Key words: Miconia, Triterpene Acids, Antibacterial Activity, Oral Pathogens

Introduction

Dental caries is a infectious disease caused by cariogenic bacteria and many approaches have been adopted to prevent them, such as elimination of the cariogenic bacteria, inhibition of bacterial plaque formation, increase in teeth resistance, and diet modification (Hamada and Slade, 1980; Tsuchya *et al.*, 1994).

Extensive efforts have been made towards the search for antibacterial substances that could eliminate the causative agents of caries. Natural products have been used for thousands of years in folk medicine for several purposes. Many plant extracts as well as isolated compounds have been shown to display anticariogenic potential, therefore attracting much interest as alternatives to synthetic chemical compounds when it comes to caries prevention (Hwang *et al.*, 2000; Koo *et al.*, 2003; Park *et al.*, 2003; Yatsuda *et al.*, 2005).

Miconia, a genus with approximately 1,000 species, belongs to the Melastomataceae family (Renner, 1993; Judd and Skean Jr., 1991). Previous studies on *Miconia* species have shown the presence of triterpenes (main constituents), as well as coumarins and benzoquinones (Lowry, 1968; Macari *et al.*, 1990; Chan *et al.*, 1992; Gunatilaka *et al.*, 2001), in these plants. Biological assays undertaken in our laboratory demonstrated that crude extracts obtained from *Miconia* species and isolated triterpene acids exhibited several biological activities such as antimicrobial, analgesic, antimutagenic and trypanocidal effects (Cunha *et al.*, 2003, 2006; Spessoto *et al.*, 2003; Celotto *et al.*, 2003; Vasconcelos *et al.*, 2006; Resende *et al.*, 2006).

As part of our ongoing research on the biological activities of Brazilian plants and natural active compounds (Da Silva Filho *et al.*, 2004; Neto *et al.*, 2005; Silva *et al.*, 2007), and because reports

documenting the antimicrobial activity of triterpene acids against oral pathogens have been scarce (Li *et al.*, 1997; Kim *et al.*, 1999), the aim of this work was to evaluate the *in vitro* antibacterial activity of triterpene acids and semi-synthetic derivatives against oral pathogens, as well as to discuss some aspects related with the structure-activity relationship.

Materials and Methods

Isolation of the triterpene acids

The triterpene acids were isolated from methylene chloride extracts of *Miconia* species according to Table I. The mixture of ursolic acid and oleanolic acid was purified by HPLC (Cunha *et al*., 2003; Vasconcelos *et al.*, 2006).

Preparation of ursolic acid derivatives

In order to obtain some triterpene acid derivatives, ursolic acid (**1**; 50 mg) was treated with an excess of acetic anhydride in pyridine to give the C-3 acetoxy derivative (45 mg) (**1a**). In another preparation, ursolic acid (about 50 mg) was treated with CH_2N_2 in Et_2O, yielding the respective C-28 methyl ester derivative (40 mg) (**1b**). The potassium salt of ursolic acid (**1c**) was prepared according to Kashiwada *et al.* (2000). A solution of ursolic acid (20 mg) was treated with 2% KOH in Me_2CO/H_2O (1:1), affording 15 mg of potassium ursolate (**1c**) after purification.

Microorganisms

The following microorganisms were used in this study: *Enterococcus faecalis* (ATCC 4082), *Streptococcus salivarius* (ATCC 25975), *Streptococcus mitis* (ATCC 49456), *Streptococcus mutans* (ATCC 25275), *Streptococcus sobrinus* (ATCC 33478), and *Streptococcus sanguinis* (ATCC 10556). All strains were acquired from the American Type Culture Collection.

Antimicrobial assay

The minimum inhibitory concentration (MIC) values of the triterpene acids and ursolic acid derivatives were determined in triplicate by using the broth microdilution method (Andrews, 2001). The samples were dissolved in DMSO at 1 mg/mL, and were then diluted in tryptone soya broth to achieve concentrations in the range 300 to 20 μg/mL. The final DMSO content was 10% (v/v), and this solution was used as negative control. The inoculum was adjusted to each organism to yield a cell concentration of 10^8 colony forming units (CFU/mL). One inoculated well was included to control the adequacy of the broth for organism growth. One non-inoculated well, free of antimicrobial agent, was also included to assure medium sterility. Chlorhexidine was used as positive control. The microplates (96-well) were incubated at 37 °C for 24 h. After that, resazurin (30 μL) in aqueous solution (0.01%) was added to the microplates, to indicate the microorganism viability (Palomino *et al*., 2002). The MIC was determined as the lowest concentration of the compound capable of inhibiting microorganism growth.

Results

The chemical structures of the evaluated compounds are displayed in Fig. 1. Their effects on the growth of the selected cariogenic bacteria are shown in Table II. All the isolated triterpene acids and semi-synthetic derivatives displayed growth inhibitory activity against the selected oral pathogens. The best MIC values were obtained for ursolic acid (**1**) and oleanolic acid (**2**). The MIC values of these pure triterpene acids ranged from 30 μg/

Table I. Summary of the isolation of the triterpene acids from *Miconia* species.

Triterpene acid	Name of the plant	Reference
Mixture of ursolic acid (**1**) and oleanolic acid (**2**)	*M. fallax* and *M. albicans*	Cunha *et al*., 2003 Vasconcelos *et al*., 2006
Sumaresinolic acid (**3**)	*M. fallax* and *M. stenostachya*	Cunha *et al*., 2003
Gypsogenic acid (**4**)	*M. stenostachya*	Cunha *et al*., 2003
Mixture of maslinic acid (**5**) and 2α-hydroxyursolic acid (**6**)	*M. sellowiana*	Cunha *et al*., 2006

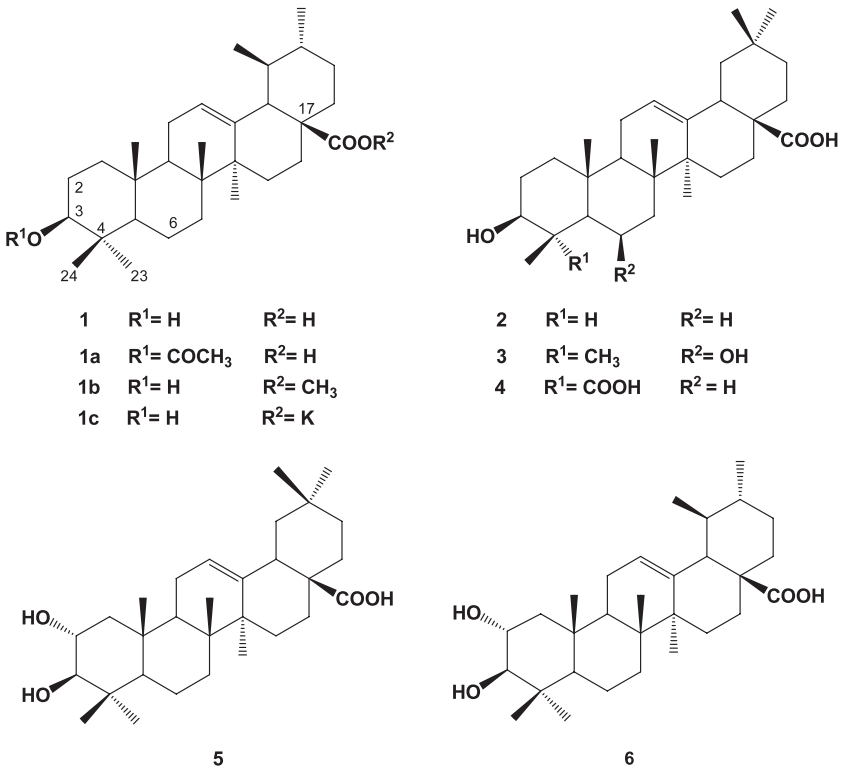

Fig. 1. Chemical structures of the triterpene acids and semi-synthetic derivatives evaluated for antibacterial activity against oral pathogens in this work.

Table II. Minimum inhibitory concentration values against oral pathogens obtained for triterpene acids and ursolic acid derivatives.

Compound	Microorganism					
	E. faecalis	*S. salivarius*	*S. sanguinis*	*S. mitis*	*S. mutans*	*S. sobrinus*
1	50	50	50	50	80	50
1a	200	90	100	50	200	90
1b	200	80	80	60	200	90
1c	100	40	60	70	80	60
2	40	30	60	40	70	50
3	200	70	80	40	200	90
4	200	70	60	50	200	100
1+2	80	60	70	30	90	80
5+6	200	80	70	60	200	200
Control	3.0	0.9	2.0	3.0	3.0	0.8

Minimum inhibitory concentration values are expressed in μg/mL. Positive control: 0.12% chlorhexidine gluconate.

mL to 80 μg/mL. Among all the microorganisms, the pathogen *S. mitis* was the most susceptible to the evaluated compounds. The majority of the compounds displayed lower inhibitory activity against the microorganisms *S. mutans* and *E. faecalis*.

Discussion

The fact that nearly similar MIC values were obtained for compounds **1** and **2** is related to their chemical structure. Because they are isomers, they differ only in the position of a methyl group. How-

ever, a mixture of **1** and **2** displayed lower inhibitory activity against the cariogenic bacteria.

The compounds sumaresinolic acid (**3**) and gypsogenic acid (**4**) displayed antimicrobial activity against all the tested bacteria, but demonstrated a lower inhibition level when compared with compounds **1** and **2**. The presence of a hydroxy group at C-6 in compound **3** and of an additional carboxy group at C-23 in compound **4** could not able to increase the inhibition level. This suggests that the free hydroxy group at C-3, as well as the carboxy group at C-17 may contribute to the inhibitory activity of the structurally related triterpene acids.

The MIC values obtained for the mixture of maslinic acid (**5**) and 2α-hydroxyursolic acid (**6**) are lower than that of the mixture of **1** and **2**, showing that the presence of a hydroxy group at C-2 is not relevant for the antibacterial activity.

The MIC values obtained for the semi-synthetic derivatives **1a**, **1b**, **1c** prepared from ursolic acid (**1**) were lower than that obtained for the starting material, reinforcing the fact that the hydroxy group and the carboxy group attached to carbon atoms 3 and 17, respectively, are important for the antimicrobial activity.

In summary, the present study showed the growth inhibitory activity of some triterpene acids against oral pathogens. These triterpene acids could be useful for the further development of new agents that could be used to reduce both dental caries and plaque formation.

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