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


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Triterpene acids (ursolic, oleanolic, 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 an 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 (Rennert, 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, anti-mutagenic 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...
Antibacterial Activity of Triterpene Acids

L. C. Scalon Cunha et al. · 669

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 CH₂N₂ in Et₂O, 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 Me₂CO/H₂O (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 mutis (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 10⁸ 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/
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.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Microorganism</th>
<th>E. faecalis</th>
<th>S. salivarius</th>
<th>S. sanguinis</th>
<th>S. mitis</th>
<th>S. mutans</th>
<th>S. sobrinus</th>
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<td>3.0</td>
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<td>0.8</td>
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</table>

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 gypsogenetic 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.

Acknowledgement

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