

Antimicrobial activity of isolated compounds and semisynthetic derivatives from *Miconia ferruginata*

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Abstract

This study evaluated the antimicrobial activity of isolated compounds and semisynthetic derivatives from *Miconia ferruginata* (Melastomataceae) against five microorganisms: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6623), *Pseudomonas aeruginosa* (ATCC 15442), and *Candida albicans* (ATCC 10231). The isomeric mixture of ursolic and oleanolic acids was active against *S. aureus* (MIC = 250 µg mL⁻¹) and against *E. coli*, *B. subtilis*, and *P. aeruginosa* (MIC = 500 µg mL⁻¹). The flavone 5,6,7-trihydroxy-4'-methoxyflavone and the methyl esters, semisynthetic derivatives of a mixture of ursolic and oleanolic acids, showed no activity against the tested microorganisms. These results suggest that the carboxyl group present in the triterpenes may contribute to antimicrobial activity.

Keywords: Melastomataceae, Oleanolic acid, , triterpenes, ursolic acid.

Atividade antimicrobiana de compostos isolados e derivados semissintéticos de *Miconia ferruginata*

Resumo

Este estudo descreve a avaliação da atividade antimicrobiana de compostos isolados e derivados semissintéticos de *Miconia ferruginata* (Melastomataceae) contra cinco microorganismos: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6623), *Pseudomonas aeruginosa* (ATCC 15442) and *Candida albicans* (ATCC 10231). A mistura isomérica dos ácidos ursólico e oleanólico foi ativa contra *S. aureus* (CIM = 250 µg mL⁻¹) e contra *E. coli*, *B. subtilis* e *P. aeruginosa* (CIM = 500 µg mL⁻¹). A flavona 5,6,7-trihidroxi-4'-metóxi-flavona e os ésteres metílicos, derivados semissintéticos da mistura dos ácidos ursólico e oleanólico, não apresentaram atividade contra os microorganismos testados. Estes resultados sugerem que o grupo carboxila presente nos triterpenos podem contribuir para a atividade antimicrobiana.

Palavras-chave: Melastomataceae, Ácido oleanólico, triterpenos, ácido ursólico.

Introduction

Infectious diseases are a significant cause of morbidity and mortality worldwide. On account that resistant microorganisms can be selected by antibiotics, attention has been paid to discover new and efficient “smart” antimicrobial drugs to counter the spread of resistance to current antibiotics (Heinemann, 2001). There is also growing interest in studying alternative antimicrobial agents, e.g., antibacterial peptides produced by microorganisms (Liu, Ding, Shen and Zhu, 2019) and plant-derived compounds (Barbieri *et al.*, 2017).

Antimicrobials of plant origin are effective in the treatment of infectious diseases and can alleviate many of the side effects often associated with synthetic antimicrobials (Savant, Venkatesh, Mannasaheb and Joshi, 2014). There are several major groups of antimicrobial compounds of plant origin: phenolics and polyphenols (including simple phenols and phenolic acids, quinines, flavones, flavonoids and flavonols, tannins and coumarins), terpenoids, essential oils, alkaloids, lectins, and polypeptides (Wolska, Grudniak, Fiecek, Kraczkiewicz-Dowjat and Kurek, 2010).

Ethnobotanical studies reported varied popular uses of species of the genus *Miconia* (Boscolo and Valle, 2008), although several plants of this genus are still poorly studied. In this context, this is the first study reporting the antimicrobial activity of isolated compounds from *M. ferruginata*.

Belonging to the family Melastomataceae (Renner, 1993), *Miconia* is a genus of approximately 1050 species (Goldenberg, Penneys, Almeda, Judd and Michelangeli, 2008), occurring in tropical America. Biological activities reported for *Miconia* species mainly include antimicrobial (Cunha *et al.*, 2010), analgesic and anti-inflammatory (Vasconcelos *et al.*, 2006), antimutagenic (Gontijo *et al.*, 2019), antitumoral (Cunha *et al.*, 2008), trypanosomicidal (Cunha *et al.*, 2006), leishmanicidal (Peixoto *et al.*, 2011), and insecticidal (Cunha *et al.*, 2017). The current study was carried out to determine the *in vitro* antimicrobial activity of isolated compounds and semisynthetic derivatives from *Miconia ferruginata* DC.

Materials and Methods

Plant material

Miconia ferruginata leaves were collected at the Cerrado of the Universidade Estadual de Goiás, Anápolis city, Goiás State, Brazil. Voucher specimens (5794) have been deposited in the Herbarium of the same University.

Compounds extraction and isolation

In previous work, Cunha *et al.* (2017) isolated a mixture of ursolic (1) and oleanolic (2) acids and the flavone 5,6,7-trihydroxy-4'-methoxyflavone (3) from the ethanolic extract of *M. ferruginata* leaves. The mixture of ursolic and oleanolic acid was converted to their methyl esters, methyl ursolate and methyl oleanolate, (1a) and (2a), respectively, by the action of diazomethane (Leonard, Lygo and Procter, 1995), aiming to verify the structure-activity relationship of the molecules. The purity of compounds was determined by TLC and analyzed from their ^1H NMR spectrum. No contamination or by-products were found.

Antimicrobial assay

Antimicrobial assays were performed using the broth microdilution technique proposed by the Clinical and Laboratory Standards Institute (CLSI) protocols M7-A6 and M27-A2 (adapted) for determining the MIC (lowest concentration able to inhibit the growth of microorganisms). All MIC determinations were performed in triplicate in 96-well microplates. The following microorganisms belonging to the American Type Culture Collection were used: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6623), *Pseudomonas aeruginosa* (ATCC 15442), and *Candida albicans* (ATCC 10231). The stock solutions of tested samples (mixture of 1 and 2, mixture of 1a and 2a, 3) were prepared in Eppendorf vials solubilizing 1 mg of sample in 40 μL of DMSO. These solutions were diluted in 960 μL of Mueller-Hinton broth for bacteria or Sabouraud broth for testing with yeast. From this solution the final concentrations ranged from 7.81 to 500 $\mu\text{g mL}^{-1}$. The inocula 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, 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 with Mueller-Hinton agar for growing bacteria and Sabouraud agar for growing fungus. The Petri dishes were incubated at 37 °C for 48 h, being verified the presence/absence of microbial colonies. After preparation of subcultures, 15 μL of 0.01% resazurin in sterile aqueous solution were added to each well of the microplates. Reading was performed after 4 h of reincubation. Thus, in each extract, it was possible to determine the lowest concentration able to inhibit the growth of microorganisms through indicators in diluted solution. All tests were performed with negative control (DMSO), positive control (vancomycin chlorhydrate, tetracycline chlorhydrate, and nystatin), microorganism growth control, and sample precipitation control, avoiding possible false-negative or false-positive results.

Results and Discussion

The chemical study of the ethanol extract of *M. ferruginata* leaves resulted in the identification of the triterpenes ursolic acid (1) and oleanolic acid (2) (mixture) and the flavone 5,6,7-trihydroxy-4'-methoxyflavone (3), in addition to the semisynthetic derivatives methyl ursolate (1a) and methyl oleanolate (2a) (mixture) (Figure 1).

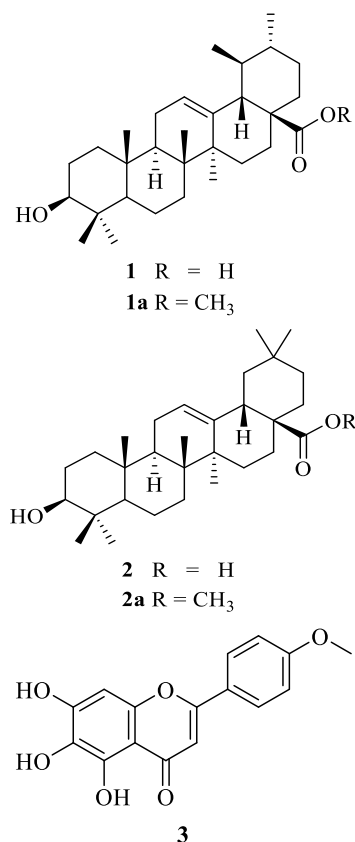


Figure 1. Chemical structures of isolated compounds and semisynthetic derivatives from *M. ferruginata*.

The mixture of ursolic and oleanolic acids showed inhibition against *B. subtilis*, *E. coli*, *P. aeruginosa* (MIC = 500 µg mL⁻¹), and *S. aureus* (MIC = 250 µg mL⁻¹). There was no activity against *C. albicans* in the tested concentration (Table 1).

Table 1. Minimum inhibitory concentration (MIC in µg mL⁻¹) values obtained for isolated compounds and derivatives from *M. ferruginata*.

Sample	<i>B. subtilis</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
1 + 2	500	500	250	500	> 500
1a + 2a	> 500	> 500	> 500	> 500	> 500
3	> 500	> 500	> 500	> 500	> 500
Positive Control	0.048*	3.125**	0.750**	1.562**	6.250***

*Vancomycin chlorhydrate, **Tetracycline chlorhydrate, ***Nystatin.

Methyl esters derivatives were also tested for their in vitro antimicrobial activity to evaluate structure-activity relationships. Introduction of the methyl group into the C-28 of the triterpenes led to disappearance of the antimicrobial activity. For the methyl esters 1a and 2a in mixture, there was neither antibacterial activity against the strains tested, nor antifungal activity against *C. albicans*. It is suggested that the carboxyl group may contribute to inhibitory activity. The flavone 5,6,7-trihydroxy-4'-methoxyflavone (3) did not showed antimicrobial activity.

Previous phytochemical investigations of *Miconia* species resulted in isolation of triterpenes (Cunha *et al.*, 2017), flavonoids (Lima *et al.*, 2018), tannins (Rodrigues *et al.*, 2011), and quinone compounds (Viegas *et al.*, 2019). The antimicrobial activity of extracts from *Miconia* species has been reported in the literature to be active against several microorganisms (Gontijo *et al.*, 2019). Furthermore, the antimicrobial properties of several triterpene acids have also been studied (Cunha *et al.*, 2010).

Triterpene acids isolated from *Miconia* species along with a mixture of those triterpenes, as well as semisynthetic derivatives, were evaluated by Cunha *et al.* (2007) against *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 triterpenes ursolic, oleanolic, gypsogetic, and sumaresinolic acids, along with a mixture of ursolic and oleanolic acids, as well as ursolic acid derivatives, displayed activity against all the tested bacteria, showing that they are promising antiplaque and anticaries agents (Cunha *et al.*, 2007).

In addition to antimicrobial activity, triterpenes isolated from *Miconia* species have been evaluated for other biological activities. Ursolic and oleanolic acids isolated from the crude methylene chloride extract of *Miconia albicans* aerial parts exhibit anti-inflammatory and analgesic activities (Vasconcelos *et al.*, 2006). In a study of the trypanocidal activity of triterpene acids isolated from *Miconia sellowiana* and *Miconia ligustroides* species, Cunha *et al.* (2006) showed that ursolic and oleanolic acids were the most active against the trypomastigote blood forms of *Trypanosoma cruzi*. Peixoto *et al.* (2011) evaluated the antileishmanial activity of the crude hydroalcoholic extract of *Miconia langsdorffii*. The fractionation of this extract led to identification of the

This antimicrobial activity is not so intense compared with antimicrobial drugs that are used clinically. However, it has been reported that ursolic and oleanolic acids are not so toxic (Liu, 1995) and possess antimicrobial activity against some resistant bacteria (Jesus *et al.*, 2015).

triterpenes ursolic and oleanolic acids as the major compounds in the fraction that displayed the highest activity. These compounds gave IC₅₀ values of 360.3 µM and 439.5 µM, respectively. In addition, a mixture of the triterpenes displayed increased antileishmanial activity, with an IC₅₀ of 199.6 µM (Peixoto *et al.*, 2011).

Studies to identify the cellular targets and molecular mechanisms of ursolic and oleanolic acids action were initiated a few years ago and it has already been demonstrated that both acids influence bacterial gene expression, the formation and maintenance of biofilms, cell autolysis, and peptidoglycan turnover. Before these compounds can be used clinically as antimicrobial agents, further extensive studies are required to determine their cytotoxicity and the optimum mode of their application (Wolska *et al.*, 2010).

Conclusion

The present study shows that the isomeric mixture of ursolic and oleanolic acids isolated from *M. ferruginata* leaves was active against *S. aureus*, *E. coli*, *B. subtilis*, and *P. aeruginosa*. However, for the methyl esters derivatives methyl oleanolate and methyl ursolate, and for the flavone 5,6,7-trihydroxy-4'-methoxyflavone, there was neither antibacterial activity against the strains tested, nor antifungal activity against *C. albicans*. It is suggested that the carboxyl group present in the triterpenes may contribute to antimicrobial activity.

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