

# ANTIBACTERIAL ACTIVITY OF *IBICELLA LUTEA* GLYCOSIDES

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## ABSTRACT

In spite of the great advances in chemotherapeutics, infectious diseases are still one of the leading causes of death in the world. Amongst the most problematic clinically relevant pathogens at present, methicillin-resistant *Staphylococcus aureus* (MRSA) ranks as one of the most difficult bacteria to treat. Interest in plants with antimicrobial properties has revived as a consequence of current problems associated with the use of antibiotics.

The objective: was to evaluate the antibacterial activity of a compound isolated from *Ibicella lutea* (Lindl.) Van Eselt. (Martyniaceae) and some of its derivatives, against methicillin-sensitive and MRSA.

Compound **1** was isolated and identified as previously described and minimal inhibitory concentration (MIC) of **1** and simple analogs were determined by the microdilution technique.

Compound **1** (11-hydroxystearic acid 11-*O*-(6-*O*-acetyl- $\beta$ -D-glucopyranoside) showed a MIC of 0.06 mM for *S. aureus* ATCC 6538p. All the synthetic analogs and precursors presented higher MIC values. Compound **1** showed antimicrobial activity against different strains of MRSA.

These results suggest that the glycosidic and acidic moieties are necessary for the antimicrobial activity under study.

*Keywords:* Martyniaceae, *S. aureus*, MRSA

## RESUMEN

A pesar de los grandes avances en la quimioterapia de las enfermedades infecciosas estas están lejos de ser controladas y más aún erradicadas y son aun una de las principales causas de muerte en el mundo. En especial *Staphylococcus aureus* metilino-resistente (MRSA) se ha mostrado como una bacteria extremadamente problemática desde el punto de vista terapéutico.

A consecuencia de los problemas encontrados en la terapéutica con antibióticos, existe hoy en día un renovado interés en productos naturales antimicrobianos.

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En este trabajo se presentan los resultados del estudio de la actividad antibacteriana de compuestos aislados de *Ibicella lutea* (Lindl.) Van Eselt. (Martyniaceae) y algunos derivados sencillos del mismo contra cepas sensibles y resistentes de *Staphylococcus aureus*.

El compuesto **1** fue aislado e identificado según las técnicas anteriormente descritas y la concentración mínima inhibitoria (MIC) de **1** y sus análogos fue determinada por una técnica de microdilución.

El compuesto **1** (11-hidroxiestearil 11-*O*-(6-*O*-acetil- $\beta$ -D-glucopiranosido) mostró una MIC de 0.06 mM para *S. aureus* ATCC 6538p. También mostró actividad antibacteriana contra diferentes cepas de MRSA mientras que todos los análogos de **1** tuvieron MICs mayores que el producto natural. Estos resultados sugieren que tanto la función ácido como la estructura glicosídica son necesarias para la actividad.

*Palabras clave:* Martyniaceae, *S. aureus*, MRSA

## INTRODUCTION

Infectious diseases are still one of the leading causes of death in the world, in spite of the great advances in chemotherapeutics. The World Health Organization (2003) states that infectious and parasitic diseases accounted for nearly 11 million out of the total of 57 million deaths in 2003.

Although there are a great array of antibacterial and antifungal drugs in clinical use, the increase of resistant organisms sometimes makes them ineffective or allows disease recurrence (Michel and Gutmann, 1996).

Among the most problematic clinically relevant pathogens at present, methicillin-resistant *Staphylococcus aureus* (MRSA) ranks as one of the most difficult bacteria to treat (Cosgrove *et al.*, 2003).

The use of higher plants and preparations made from them to treat infections is an age-old practice in a large part of the world population, especially in developing countries, where traditional medicine is used for a variety of diseases. Interest in plants with antimicrobial properties has revived as a consequence of current problems associated with the use of antibiotics (Cherigo *et al.*, 2009; Po Shiu and Gibbons, 2006).

*Ibicella lutea* (Lindl.) Van Eselt. (Martyniaceae) is the sole species of the genus

*Ibicella* from the small Martyniaceae family comprising just 3 genera (*Martynia*, *Proboscoidea* and *Ibicella*) with 13 species (Van Eseltine, 1929; Bretting, 1984).

In Uruguay, where the plant grows freely, it is called "Cuerno del Diablo" (Devil's horn) and its infusion is used in popular medicine as an antimicrobial for the treatment of eyes and skin infections (Alonso Paz *et al.*, 1995 and references therein).

In previous work we studied the antimicrobial activity of aqueous and ethanol extracts of several Uruguayan medicinal plants. We found that *I. lutea* indeed has an important activity, deserving further study (Olano *et al.*, 1996), and we isolated one of the active principles, 11-hydroxystearic acid 11-*O*-(6-*O*-acetyl- $\beta$ -D-glucopyranoside) (Cerdeiras *et al.*, 2000).

In this paper we report the antibacterial activity of this compound along with some of its derivatives against methicillin-sensitive and MRSA.

## MATERIALS AND METHODS

### General analytical methods

Gas chromatography (GC) analysis was performed in a Shimadzu GC 14 apparatus with an SE-52 column using a temperature program from 100° C to 280° C with a 5°

C min<sup>-1</sup> gradient. A Bruker micrOTOF-Q-TOF with ESI source in positive mode was used for mass spectra (MS) and a Shimadzu QP 5050 with a SE 52 column was used for the GC-MS analysis.

<sup>1</sup>H NMR spectra were obtained at 400MHz and <sup>13</sup>C NMR spectra at 100MHz on a Bruker Advance DPX 400 spectrometer, using CD<sub>3</sub>OD or CDCl<sub>3</sub> as solvents, and TMS ( $\delta$ H 0.00) and acetone ( $\delta$ C 31.00) as references. Two dimensional (different H, H-COSY, H, C-COSY, HMBC, HMQC and NOESY) experiments were carried out with programs available for the Bruker software.

#### Plant material

Plant material was collected in Pajas Blancas, 6 Km outside Montevideo at 34° 51' 58.33" S, 56° 20' 05.38" W. The material was identified by Lic. E. Alonso Paz, Botany Department, Facultad de Química, UDELAR and voucher specimens (Leg. Alonso Paz et Bassagoda N° 2546) were kept in the MVFQ Herbarium, Montevideo.

#### Compounds

Compound **1** was isolated and identified as previously described (Cerdeiras *et al.*, 2000). Compound **2** and **3** were obtained by acetylation of **1** with Ac<sub>2</sub>O/Pyridine and mild alkaline hydrolysis respectively. Compound **4** was synthesized from  $\alpha$ -bromo-tetra-acetyl-glucose and methyl 11-hydroxyoctadecanoate by the classical Koenigs-Knorr method using Ag<sub>2</sub>O as catalyst (Talley, 1963). The resulting compounds were identified by different GC-MS, HRMS and NMR techniques. Compounds **5** (12-hydroxy-9-octadecenoic acid) and **6** (methyl 11-hydroxyoctadecanoate) were purchased from Sigma.

#### Microbiological analysis

The strains used were *Staphylococcus aureus* ATCC 6538p and the clinical multiresistant isolates *S. aureus* CCMC (Colección Cátedra Microbiología Clínica) 15027, 16369 and 19016

Minimal inhibitory concentration (MIC) was determined for the extracts that inhibited growth by the microdilution technique according to Elloff (1998) using 100  $\mu$ l of Mueller-Hinton broth (Difco), 100  $\mu$ l of two-fold dilutions of the extracts (10 mgmL<sup>-1</sup>) and 10  $\mu$ l of a suspension (10<sup>8</sup>/mL) of the microorganisms. The trays were incubated (24 h, 37C) and developed with *p*-iodo-nitrotetrazolium violet (INT) (Sigma) 0.1% solution. Bacterial growth was indicated by development of a red formazan color. Gentamicin was used as control.

## RESULTS AND DISCUSSION

Compound **1** had previously demonstrated very promising antimicrobial activity (Cerdeiras *et al.*, 2000) against reference strains, in particular against susceptible *S. aureus* ATCC 6538p.

To see if the antimicrobial activity could be enhanced by some simple structural modifications, we prepared derivatives **2**, **3** and **4** (Fig. 1). We also tested the free acid **5** and the methyl ester **6**.

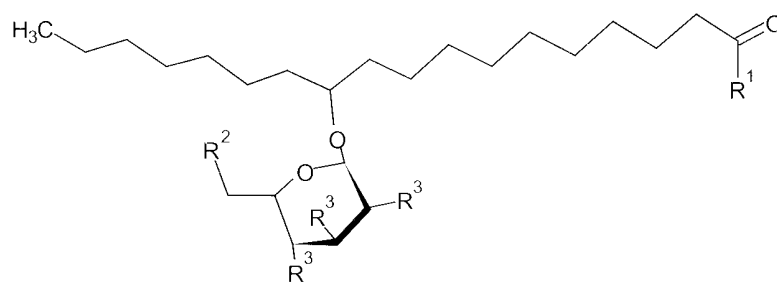
The results of the antibacterial activity of the compounds against *S. aureus* ATCC 6538p are depicted in Table 1.

The analysis of these results suggests that both the free acid function and the glycosidic moiety are indispensable for the activity. Compounds **4** and **6** which are methyl esters lack any antibacterial activity. Also the free acid **5** has a MIC that is more than 15-fold greater (on a mol/L basis) than compound **1**.

When MICs for compounds **1**, **2** and **3** are compared it is also clearly seen that the natural monoacetyl compound is 10 times more active than the tetra-acetyl and desacetyl derivatives.

More studies are needed to determine if this is due to solubility properties of the molecules or to a specific interaction with the bacterial target.

Compound **1** was also tested against *S.*



R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	Compound
OH	AcO	OH	<b>1</b>
OH	AcO	AcO	<b>2</b>
OH	OH	OH	<b>3</b>
OMe	AcO	AcO	<b>4</b>

**Figure 1.** Structural formulas of tested compounds.

**Table 1.** Antimicrobial activity of the tested compounds against *S. aureus* 6538p.

Compound	MIC $\mu\text{g/mL}$	MIC (mM)
1	31.3	0.06
	312.5	0.50
3	225.0	0.49
4	> 1000	--
12-hydroxy octadecenoic acid (5)	300.0	1.01
Methyl 11-hydroxyoctadecanoate (6)	>1000	--

MIC = Minimal inhibitory concentration

**Table 2.** Phenotypic susceptibility profile of methicillin-resistant strains.

Strain	P	Ox	Er	Cc	Gm	Cip	Ra	SxT	Te	Va	<i>mecA</i>
16369	R	R	S	ND	S	ND	S	S	S	S	ND
19016	R	R	ND	R	R	R	R	R	R	S	+
15027	R	R	ND	ND	R	ND	S	R	R	S	+

R: resistant, S: sensitive, ND: not determined. P: Penicillin, Ox: Oxacillin, Er: Erythromycin, Cc: Clindamicin, Gm: Gentamicin, Cip: Ciprofloxacin, Ra: Rifampicin, SxT: Sulfamethoxazole/trimethoprim, Te: Tetracycline, Va: Vancomycin.

*aureus* clinical isolates that showed different antibiotic resistance profiles, but were all MRSA. The phenotypic susceptibility profile of these strains is shown in Table 2. Strain 16369 is a hospital acquired isolate and strains 15027 and 19016 are community acquired infection isolates. The MICs obtained with these strains were 250, 500 and 62.5 µg/mL respectively.

## CONCLUSIONS

The present work clearly shows that higher plants are still a primary source in the

search of lead molecules for novel antimicrobial compounds.

The original compound **1** was the one that presented the highest activity against the *S. aureus* ATCC 6538p strain. But, more studies are needed to determine if this action is due to solubility properties of the molecule or to a specific interaction with the bacterial target.

Even though the MIC results obtained with the MRSA strains were relatively high, compound **1** is still of interest as a lead compound given that it is a simple and easily synthesized molecule.

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