

Full Length Research Paper

Antimicrobial activity of artocarpesin from *Artocarpus heterophyllus* Lam. against methicillin-resistant *Staphylococcus aureus* (MRSA)

Navarro-García Víctor Manuel^{1*}, Salinas-Sánchez David Osvaldo^{1,2},
Rodríguez-Flores Tannia Alejandra¹, Abarca-Vargas Rodolfo^{1,3} and Aldana-Llanos Lucila⁴

¹Laboratorio de Microbiología. Centro de Investigación Biomédica del Sur-Instituto Mexicano del Seguro Social (CIBIS-IMSS), Xochitepec, Morelos, Mexico.

²Departamento de Recursos Naturales del Centro de Investigación en Biodiversidad y Conservación (CIByC), Universidad Autónoma del Estado de México (UAEM), Cuernavaca, Morelos, Mexico.

³Laboratorio de Farmacología. Centro de Investigación Biomédica del Sur-Instituto Mexicano del Seguro Social (CIBIS-IMSS), Xochitepec, Morelos, Mexico.

⁴Laboratorio de Entomología, Centro de Desarrollo de Productos Bióticos (CENPROBI), Instituto Politécnico Nacional (IPN), Yauatepec, Morelos, Mexico.

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We evaluated the antibacterial activity of the organic extracts (*n*-hexane, acetone and methanol) from the fruit of *Artocarpus heterophyllus* Lam. against *Staphylococcus aureus*, (methicillin-susceptible *Staphylococcus aureus* [MSSA]). The extract that demonstrated the greatest activity was that of acetone, with a minimum inhibitory concentration (MIC) value of 0.375 mg/ml against *S. aureus* MSSA. The acetone extract was further fractionated by means of open-column chromatographic methods into different fractions, and it was found that the F3 fraction was the most active. An active compound against MSSA and methicillin-resistant *Staphylococcus aureus* bacteria was isolated from this fraction; this compound was identified through spectroscopic methods of ¹H and ¹³C nuclear magnetic resonance as artocarpesin.

Key words: Antimicrobial activity, artocarpesin, *Artocarpus heterophyllus*, methicillin-resistant *Staphylococcus aureus* (MRSA).

INTRODUCTION

The inadequate use and abuse of antibiotics has propitiated the appearance of bacterial resistance. At present, this resistance constitutes a worldwide problem, but this is particularly more severe in developing countries where last-generation synthetic antimicrobials are frequently not available or are not accessible for an important number of the population due to their high costs (Murphy, 1999). According to the World Health Organization (WHO), infectious diseases are found

among the main causes of mortality of up to 90% at the worldwide level (Guzmán et al., 2001). *Staphylococcus aureus* is a bacterium that causes a great variety of infections of the skin, of the soft tissues, bacteremia, endocarditis and central nervous system (CNS) and genitourinary tract infections (Gil, 2000). Many infectious diseases unfortunately remain unresolved due to the serious problems that are caused by the appearance of antibiotic-resistant mutant bacteria. Methicillin-resistant *S. aureus* (MRSA) is one of the principal bacteria that cause nosocomial infections in hospital at the world level (Takeda et al., 2000; Velazquez, 2005).

Medicinal plants constitute an important reservoir of bioactive molecules and in particular, a potential source

*Corresponding author. E-mail: vicmanchem@gmail.com. Tel: +52) (777) 361 21 55.

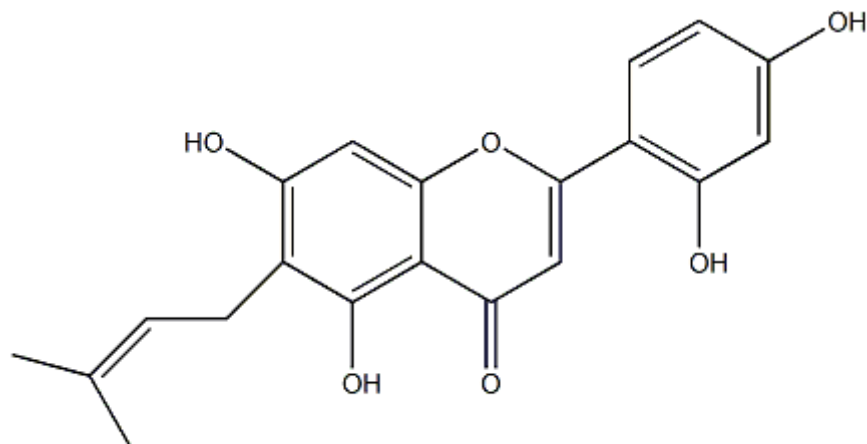


Figure 1. Active compound (artocarpesin) isolated from *A. heterophyllum*.

of anti-infectious agents (Okundane et al., 2004; Ríos and Recio, 2005). The plant *Artocarpus heterophyllum* is a tree native to India and Malaysia that was introduced into Africa by the Arabs and later into South America, and has been adapted in Mexico. In Southeast Asia, it has great commercial, nutritional and medicinal value and is cultivated mainly for its fruit, which is utilized in traditional medicine for treating anemia, asthma, wound healing, ulcers, dermatitis, diarrhea and cough (Jagtap and Bapat, 2010). Studies were performed on anti-inflammatory activity (Fang et al., 2008; Wel et al., 2005), antibacterial activity (Khan et al., 2003), antioxidant properties (Ko et al., 1998) and antidiabetic activity (Fernando et al., 1991). The chemical constituents of *Artocarpus* species have earlier been reviewed and one of the principal compounds reported of this plant are phenolic-type compounds (Hakim et al., 2006).

The objective of the present work was to investigate the antibacterial activity of the extracts (fruit) of the plant against *S. aureus* sensitivity and resistance. The compound responsible for the antibacterial activity was identified as the 5,7,2',4'-tetrahydroxy-6-(3-methylbut-3-enyl) flavone, artocarpesin (**1**) (Figure 1), by means of the direct comparison of the nuclear magnetic resonance (NMR) spectra of ^1H and ^{13}C reported by Monache et al. (1994). To our knowledge, this would be the first report on the compound (**1**) to describe the antimicrobial potential against MRSA.

MATERIALS AND METHODS

The fruit of *A. heterophyllum* was collected in October 2010 in the Mexican municipality of Cuernavaca, Morelos State. Identity of the species was determined by Macrina Fuentes, B.Sc. Biology and Margarita Avilés, B.Sc. Biology. A sample of the plant material was deposited at the Herbarium of the Instituto Nacional de Antropología e Historia de Morelos (INAHM), with registry number INAHM 2044. The fruit was cut into slices and hung in a dark room at room temperature where it was left to dry for 5 days until its

complete dehydration. The dry material (900 g) was ground utilizing an electric grinder (Pulvex Model 95).

Preparation of the extracts

The plant material was set in place for maceration in *n*-hexane during 3 days and on three occasions. The macerate was filtered to remove the solid part and the solvent was eliminated under reduced pressure in a rotavapor (Heidolph WD 4000). Later, we performed other consecutive extractions of plant material with acetone and methanol, proceeding by the way described previously, in order to obtain the corresponding extracts. The following yields were obtained: *n*-hexane, 1.66%, acetone, 5.1% and methanol, 18%.

Microorganisms

The microorganisms used in this study included the following: *S. aureus*-sensitive ATCC 6538. The bacteria of MRSA were obtained from clinical isolates from the Instituto Mexicano del Seguro Social (IMSS) Regional Hospital of Cuernavaca. The cultures were maintained in Mueller-Hinton (MH) agar (Merck, AMH) at 4°C until immediately prior to their use. The bacteria were inoculated in MH broth (Merck) at 37°C, 18 h prior to initiation of the test.

Determination of antibacterial activity

For antimicrobial evaluation of the extracts, we only used a *S. aureus* strain sensitive to the antibiotic (MSSA, ATCC 6538); for fractions and the pure product. We additionally employed Methicillin-resistant *S. aureus* (MRSA) strains. Extracts, fractions, the isolated compound and the standard antibiotic (gentamicin, penicillin and dicloxacillin) were serially diluted in microplate wells until a concentration of the extracts (0.187 - 6.0 mg/ml), fractions (6.25 - 800 µg/ml), the isolated compound (**1**), and the standard antibiotics (1.0 - 128 µg/ml) was acquired.

We utilized the serial liquid microdilution method as described by Eloff (1998). The cultures were adjusted to 10^6 colony-forming unit (CFU)/ml inoculum size, employing the standard 0.5 MacFarland scale. Five microliters of culture was added to each well, which contained culture medium and the diluted samples. The microplates were placed under incubation at 37°C for 24 h. After this incubation period, 20 µL of *p*-iodonitrotetrazolium violet (0.5 mg/ml) was added

Table 1. Minimum inhibitory concentration (MIC) values of three extracts of *Artocarpus heterophyllus* against the *Staphylococcus aureus*-sensitive strain*.

Extract	MIC (mg/ml)
<i>n</i> -Hexane	>6.0
Acetone	0.375
Methanolic	>6.0
Chloramphenicol	0.002
Oxacillin	0.002
Ampicillin	0.002

**Staphylococcus aureus* American Type Culture Collection (ATCC) 6538.

Table 2. Minimum inhibitory concentration (MIC) values of fractions, product (1) and standard antibiotics against the sensitive strain and the two Methicillin-resistant *Staphylococcus aureus**(MRSA) strains.

Parameter	MIC (mg/ml)		
	MSSA	MRSA 1	MRSA 2
Fractions			
F1	>0.200	>0.200	>0.200
F2	0.200	0.200	0.200
F3	0.050	0.100	0.050
Product: Artocarpesin	0.008	0.016	0.016
Antibiotics			
Chloramphenicol	0.001	0.004	0.004
Oxacillin*	0.002	0.032	0.128
Ampicillin*	0.002	0.064	0.128

*MIC values of oxacillin and ampicillin ≥ 4 μ L were considered resistant strains.

to each well of the microplate.

The formation of a red color is indicative of cellular viability. The MIC value was determined as the lowest concentration of the sample assayed that did not form the red color in the microplate well.

Fractioning of the acetone extract

Fractioning of the acetone extract was carried out in columns packed with silica gel 60 (Merck, 70-230 mesh) and was eluted in an *n*-hexane: acetone ascending polarity index, initiating with 100% *n*-hexane. The process was monitored by thin layer chromatography (TLC). Fractions exhibiting patterns similar to those in TLC were grouped according to their chemical content, containing three main active fractions: F1, F2 and F3. Fraction F3 (800 mg) was submitted to a second chromatographic fractioning as it comprised the fraction that demonstrated greatest activity, using an isocratic elution system: *n*-hexane: ethyl acetate: acetone at a ratio of 8:1:1, from which we obtained eight fractions (A - H). From fractions E - G, we isolated and purified the active compound (1).

obtained from *A. heterophyllus* against the MSSA strain, in which the acetone extract demonstrated important biological activity with an MIC value of 0.375 mg/ml; the remaining two extracts did not demonstrate antimicrobial activity. In Table 2, the antimicrobial activity is depicted of the three most active fractions of the acetone-extract chromatographic column, in which we were able to observe that fraction F3 demonstrated the greatest antimicrobial activity against the MSSA strain and those of MRSA. From this fraction, we isolated and purified the artocarpesin compound (1), which demonstrated possession of important antimicrobial activity against the MSSA strain and those of MRSA. Moreover, the product responsible for the activity exhibited by the F3 fraction was identified as the prenylated flavone 5,7,2',4'-tetrahydroxy-6-(3-methylbut-3-nyl) (artocarpesin) (1). This flavone was identified by direct comparison with the spectroscopic data of ^1H and ^{13}C NMR with those described by Monache et al. (1994).

RESULTS

Table 1 shows the MIC values of the three extracts

DISCUSSION

In our research, we found that the active extract was that

of acetone and from this, we isolated a flavone (1) as responsible for the antimicrobial activity. This compound is capable of inhibiting the growth of the bacteria assayed in this study, with an MIC value of 8 µg/ml against the MSSA strain and of 16 µg/ml against the two MRSA strains. These results are in agreement with the previous report of Sato et al. (2000) in which the authors found that apigenin- and luteolin-type flavones present antimicrobial activity, with apigenin being the more active of the two, in an MIC range of 3.9-15.6 µg/ml against several MRSA strains.

In 1996, Tsuchiya et al. describe a chemical study of the anti-MRSA structure-activity relationship of flavones, which demonstrated that substitutions in the A and B rings of the flavones are important in the expression of antimicrobial activity. They (Tsuchiya et al., 1996) described that dihydroxylation in positions 2',4' - or 2',6' of the B ring and dihydroxylation in positions 5,7 of the A ring are of significant importance for anti-MRSA activity; additionally, the substitution of certain aliphatic groups in position C6 or C8 increase this activity. The artocarpesin compound (1) reported in this research complies with all of the chemical characteristics described by these authors, which reaffirms and explains the anti-MRSA activity of compound (1) shown in this research.

Our results therefore suggest that artocarpesin and, certainly, related flavonoids are compounds with the antimicrobial potential of usefulness in the treatment of infections produced by MRSA.

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