Anti-staphylococcal activity of Syagrus coronata essential oil

Biofilm eradication and in vivo action on Galleria mellonela infection model

dos Santos, Bruno Souza; Macedo Bezerra Filho, Clóvis; Adelson Alves do Nascimento Junior, José; Brust, Flávia Roberta; Bezerra-Silva, Patrícia Cristina; da Rocha, Suyana Karoline Lino; Krogfelt, K. A.; do Amaral Ferraz Navarro, Daniela Maria; dos Santos Correia, Maria Tereza; Napoleão, Thiago Henrique; da Silva, Luís Claudio Nascimento; Macedo, Alexandre José; da Silva, Márcia Vanusa; Guedes Paiva, Patrícia Maria

Published in:
Microbial Pathogenesis

DOI:
10.1016/j.micpath.2019.04.009

Publication date:
2019

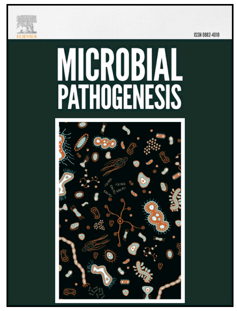
Document Version
Peer reviewed version

Citation for published version (APA):
dos Santos, B. S., Macedo Bezerra Filho, C., Adelson Alves do Nascimento Junior, J., Brust, F. R., Bezerra-Silva, P. C., da Rocha, S. K. L., Krogfelt, K. A., do Amaral Ferraz Navarro, D. M., dos Santos Correia, M. T., Napoleão, T. H., da Silva, L. C. N., Macedo, A. J., da Silva, M. V., & Guedes Paiva, P. M. (2019). Anti-staphylococcal activity of *Syagrus coronata* essential oil: Biofilm eradication and *in vivo* action on *Galleria mellonela* infection model. *Microbial Pathogenesis*, *131*, 150-157. https://doi.org/10.1016/j.micpath.2019.04.009

Anti-staphylococcal activity of Syagrus coronata essential oil: Biofilm eradication and in vivo action on Galleria mellonela infection model

Article *in* Microbial Pathogenesis · April 2019

DOI: 10.1016/j.micpath.2019.04.009



Anti-staphylococcal activity of *Syagrus coronata* essential oil: Biofilm eradication and *in vivo* action on *Galleria mellonela* infection model

Bruno Souza dos Santos, Clóvis Macedo Bezerra Filho, José Adelson Alves do Nascimento Junior, Flávia Roberta Brust, Patrícia Cristina Bezerra-Silva, Suyana Karoline Lino da Rocha, Karen Angeliki Krogfelt, Daniela Maria do Amaral Ferraz Navarro, Maria Tereza dos Santos Correia, Thiago Henrique Napoleão, Luís Claudio Nascimento da Silva, Alexandre José Macedo, Márcia Vanusa da Silva, Patrícia Maria Guedes Paiva

PII: S0882-4010(18)31395-0

DOI: https://doi.org/10.1016/j.micpath.2019.04.009

Reference: YMPAT 3481

To appear in: *Microbial Pathogenesis*

Received Date: 7 August 2018

Revised Date: 4 April 2019

Accepted Date: 5 April 2019

Anti-staphylococcal activity of *Syagrus coronata* essential oil: biofilm eradication and *in vivo* action on *Galleria mellonela* infection model

Bruno Souza dos Santos[a], Clóvis Macedo Bezerra Filho[a], José Adelson Alves do Nascimento Junior[a], Flávia Roberta Brust[b], Patrícia Cristina Bezerra-Silva[c], Suyana Karoline Lino da Rocha[c], Karen Angeliki Krogfelt[d], Daniela Maria do Amaral Ferraz Navarro[c]; Maria Tereza dos Santos Correia[a], Thiago Henrique Napoleão[a], Luís Claudio Nascimento da Silva[e], Alexandre José Macedo[b], Márcia Vanusa da Silva[a], Patrícia Maria Guedes Paiva[a,*]

[a]*Departamento de Bioquímica, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*

[b]*Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.*

[c]*Departamento de Química Fundamental, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*

[d] *Department of Virology and Microbiological Diagnostics, Statens Serum Institut, Denmark.*

[e]*Programa de Pós-Graduação em Biologia Parasitária, Universidade CEUMA, Maranhão, Brazil.*

*Corresponding author. E-mail address: ppaivaufpe@yahoo.com.br

Abstract

In this study, essential oil extracted from *Syagrus coronata* seeds (SCEO) was evaluated for antibacterial and antibiofilm activities against *Staphylococcus aureus*; in addition, *Galleria mellonella* model was used as an *in vivo* infection model. SCEO was mainly composed by fatty acids (89.79%) and sesquiterpenes (8.5%). The major components were octanoic acid, dodecanoic acid, decanoic acid and γ-eudesmol. SCEO showed bactericidal activity (minimal bactericidal concentration from 312 to 1250 μg/mL) against all tested *S. aureus* clinical isolates, which showed distinct biofilm-forming and multiple drug resistance phenotypes. SCEO weakly reduced biomass but remarkably decreased cell viability in pre-formed biofilms of *S. aureus* isolate UFPEDA-02 (ATCC-6538). Electron microscopy analysis showed that SCEO treatments decreased the number of bacterial cells (causing structural alterations) and lead to loss of the roughness in the multiple layers of the three-dimensional biofilm structure. In addition, overproduction of exopolymeric matrix was observed. SCEO at 31.2 mg/kg improved the survival of *G. mellonela* larvae inoculated with UFPEDA-02 isolate and reduced the bacterial load in hemolymph and melanization. In conclusion, SCEO is an antibacterial agent against *S. aureus* strains with different resistance phenotypes and able to disturb biofilm architecture. Our results show SCEO as a potential candidate to drug development.

Keywords: *Staphylococcus aureus*; antibiofilm; antibacterial activity; volatile oil.

1. Introduction

Antimicrobial resistance is one of the most serious public health problems, especially in developing countries where infectious diseases still represent a major cause of human mortality [1]. *Staphylococcus aureus* is highlighted as one of the major human pathogens due to its high ability to produce virulence factors that mediate evasion of immune system and host tissue damage [2–4]. Diseases caused by *S. aureus* involve skin infections (boils, folliculitis, and abscesses) and diseases with greater severity such as pneumonia, meningitis, osteomyelitis, endocarditis, bacteremia, and sepsis [5, 6]. In addition, the widespread and indiscriminate use of antibiotics has caused selective pressure favoring the development of resistant strains, such as methicillin-resistant *S. aureus* (MRSA) and other multidrug resistant phenotypes. MRSA is associated with high rates of morbidity and mortality [5–7].

As other bacteria, *S. aureus* often survive by adhering to surfaces on which they form complex structures called biofilms [2]. Biofilms are conglomerates of microbial cells protected by self-synthesized extracellular polysaccharide matrices. Bacterial biofilms are one of the most common causes of persistent infection and represents a major health problem, as it plays an important role in nosocomial infections when formed in internal medical devices such as implanted catheters, artificial heart valves, or bone and joint prostheses [8, 9]. The ability of *S. aureus* to form biofilms in implanted medical devices is an important virulence factor for this pathogen [9]. Biofilm producer strains usually exhibit increased resistance to antibiotics and are responsible for persistent infections [8, 10].

The failure of the antibiotics currently used in treating infections caused by multidrug resistant microorganisms has driven the search for new compounds and alternative treatments, particularly those involving plant-derived products such as essential oils, flavonoids and other secondary metabolites [11–13]. Essential oils are mixtures of odoriferous and volatile compounds that have been widely reported as antimicrobial agents [12, 14]. One example of essential oil bearing plant is *Syagrus coronata* (Martius) Beccari (Arecaceae, Arecoideae), popularly known as “licuri” or “ouricuri” [15].

Syagrus coronata is an edible oil crop known to produce high amount of oils, with potential use for various purposes [16, 17]. In addition, it has a number of applications in folk medicine including snakebites, ocular inflammations, mycoses, wound healing, and spinal pain treatment [18]. Various biological activities have been reported for *S. coronata* seed oil, including antibacterial and insecticidal properties [16, 19]. In addition, it has shown moisturizing property [20].

Galleria mellonella larvae (waxmoth) is an alternative model that has attracted attention due the methodological simplicity and reliability in the evaluation of infections caused by different human pathogens, in the discovery of new virulence genes, as well as in the evaluation of toxicity and efficacy of antimicrobial agents [21–23].

In the present study, *S. coronata* seed essential oil (SCEO) was evaluated for antibiotic and antibiofilm activity against *S. aureus* and *G. mellonella* was used as an *in vivo* infection model.

2. Material and methods

2.1. Plant material

Seeds of *S. coronata* were collected at the Catimbau National Park region (PARNA do Catimbau, Pernambuco, Brazil - 8° 30' 57" S, 37° 20' 59" W) in December 2015. They were dried at 30 °C in an open and airy area for three weeks. The taxonomic identification of the plant was performed by Dr. Alexandre Gomes da Silva in the herbarium of the *Instituto*

Agronômico de Pernambuco (IPA). The voucher specimen was deposited under the number 86,950. The access was recorded (AFD8A80) in the *Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional. Associado* (SisGen).

2.2. Extraction of S. coronata essential oil (SCEO)

The dried seeds (200 g) were powdered and essential oil was obtained by hydrodistillation method for 4 h in a modified Clevenger-type apparatus. The SCEO layer was separated from the hydrolate (aqueous layer), dried over anhydrous sodium sulfate, and stored in a hermetically sealed amber-glass vial at -20 ºC until required for analysis. The percentage yield of essential oil was taken as the ratio between the weight of oil obtained and the weight of seed powder. The whole procedure was repeated 3 times.

2.3. Gas chromatographic (GC) analyses

SCEO was esterified by acid catalysis with boron trifluoride (BF3) [24]. GC was performed using a Thermo Fisher Scientific (Waltham, MA, USA) Trace GC Ultra gas chromatograph equipped with a flame ionization detector (FID), a split/splitless injector and a Hamilton Bonaduz (Switzerland) HB-5 fused silica capillary column (30 m × 0.25 mm; film thickness 0.25 µm). The oven temperature was held at 40 ºC for 2 min and then increased at 4 ºC/min to 230 ºC. The injector and detector were both maintained at 250 ºC, and the essential oil solution and esterified fractions were injected in the splitless mode. Each analysis was carried out in triplicate.

GC coupled to mass spectrometry (GC-MS) was carried out using an Agilent Technologies (Palo Alto, CA, USA) series 5975C quadrupole analyzer equipped with an

Agilent J & W nonpolar DB-5 fused silica capillary column (60 m x 0.25 µm i.d.; film thickness 0.25 µm). The oven temperature was held at 60 ºC for 3 min, then increased at 2.5 ºC/min to 240 ºC and subsequently held for 10 min. The helium carrier gas flow was maintained with a constant pressure of 100 kPa, and the injector was operated at 250 ºC in the split mode (1:20). The detector temperature was 280 ºC, the ionization potential was 70 eV, and mass spectra were scanned in the range 20-350 m/z at a rate of 0.5 scans/s [25].

Individual components of SCEO were initially identified according their Retention Indices (RI), obtained by co-injection of oil samples and C_8-C_{30} n-alkanes, calculated according to the equation of van Den Dool and Kratz [26] and compared with the literature [27]. The acquired mass spectra were matched with those stored in the library of the GC-MS system (MassFinder 4 comprising NIST08 MS Library and Wiley Registry of Mass Spectral Data, 9th Edition) and with other published data. The composition of essential oil was expressed as percentages of total peak area as recorded by GC-FID.

2.4. Phenotypic characterization of S. aureus strains

2.4.1. Antibiotic susceptibility profile

Twenty *S. aureus* clinical isolates (Table 2) were obtained from the Collection of Microorganisms of the *Departamento de Antibióticos* of the *Universidade Federal de Pernambuco* (UFPEDA, WDCM0114), Brazil. *S. aureus* clinical isolates susceptibility was determined according to Kirby Bauer's disk diffusion technique [28] using the antibiotics: oxacillin, ciprofloxacin, nitrofurantoin, amikacin, gentamicin, clindamycin, chloramphenicol, tetracycline, and trimethoprim. Antibiotic susceptibility was interpreted according the Clinical and Laboratory Standards Institute [31].

The multiple antibiotic resistance (MAR) index was calculated using the formula *MAR= x/y*, where *x* is the number of antibiotics to which the isolate demonstrated resistance and *y* is the total number of antibiotics tested [29].

2.4.2. Evaluation of S. aureus biofilm formation

Biofilm formation was evaluated and quantified using a microtiterplate test [30]. Briefly, it was added 20 µL of the bacterial suspension (1.5 x 10^8 CFU/mL), 20 µL of Milli-Q water and 160 µL of brain heart infusion broth (BHI) in each well of the plate. After 24 h of incubation at 37 °C, the non-adhered cells were removed, and the biofilm was washed three times with saline solution (0.9% NaCl). Biofilms were heat-fixed at 60 ºC for 1 h and then stained with 0.4% (w/v) crystal violet for 15 min at 30 ºC. Finally, the plate was washed four times with water and the biofilm was resuspended with ethanol for 30 min. The optical density (OD) was measured at 570 nm. The biofilm production was classified according to Stepanovic et al. [30].

2.5. Determination of minimal inhibitory (MIC) and bactericidal (MBC) concentrations

Minimal inhibitory concentration (MIC) were determined by broth microdilution method. Initially, overnight bacterial culture was prepared on Mueller Hinton Agar (MHA) plates. The, a bacterial suspension at 1.5×10^8 CFU/mL was prepared in saline solution (0.9% NaCl). SCEO (0.039–10,000 µg/mL in 5% dimethyl sulfoxide, DMSO) was serially diluted in microplates containing Mueller Hinton Broth (MHB). Each well received 10 µL of bacterial suspension, except the wells used as sterility control. In negative control, it was used 5% DMSO. The plates were incubated at 37 °C and, after 24 h, wells received 20 µL of 0.01% (w/v) resazurin solution to follow bacterial growth (i.e. change of blue to pink color). After 24-h incubation, the MIC was defined as the lowest SCEO concentration that inhibited bacterial growth. Suspension from wells before the addition of resazurin were transferred to MHA plates and incubated for other 24 h. MBC was determined as the lowest SCEO concentration able to prevent bacterial growth. The MIC_{50} and MIC_{90} were determined as the MIC values that inhibits 50% and 90% of the *S. aureus* isolates (n = 20).

2.6. Biofilm eradication assays: Quantification of biofilm biomass and viability of biofilm cells

The biofilm eradication ability of SCEO was evaluated according to Zimmer et al. [32]. For this assay, the *S. aureus* UFPEDA-02 (ATCC-6538) strain was selected due to its source (wound) and high biofilm production ability. The biofilm was formed according previously described and, after 24 h of incubation at 37 °C, planktonic cells were removed and the SCEO diluted in BHI broth was added at different concentrations (156, 312, 624 and 1,248 µg/mL in 5% DMSO). The plate was incubated again at 37 °C and after 24 h the wells were washed three times with saline solution (0.9% NaCl). Adherent biofilms were heat-fixed at 60 ºC for 1 h and then stained with 0.4% (w/v) crystal violet for 15 min at 30 ºC. Finally, the plate was washed four times with water and the stained biofilm was solubilized in ethanol for 30 min. The absorbance (570 nm) was measured. Vancomycin (1 µg/mL) was used as antibiotic control.

The viability of cells within biofilms exposed SCEO was assessed using MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assay [33]. Biofilms were grown as described above and, after 24 h, it was exposed to the SCEO (156, 312, 624 and 1,248 µg/mL in 5% DMSO). After the incubation period, the content of wells was removed and the remaining biofilm was washed two times with saline. MTT solution at 0.3 mg/mL (200 µL) was added to each well and incubated for 90 min at 37 °C. The wells were then washed once with saline and the purple formazan crystals were dissolved with 200 µL of DMSO for 20 min and then the absorbance at 540 nm was measured. Vancomycin (1 µg/mL) was used as positive control.

2.7. Scanning electron microscopy (SEM)

Biofilm was grown and treated with SCEO (312 and 624 µg/mL in 5% DMSO) in 96-wells microtiter plates containing a piece of Permanox™ slide in each well (Nalge Nunc International, USA). After 48 h of incubation at 37 ºC, the slide pieces were fixed and stored in 2.5% (v/v) glutaraldehyde at -20 ºC until microscopy analysis. The samples were washed with 100 mM cacodylate buffer pH 7.2 and dehydrated in increasing concentrations of acetone. The slides were dried by the CO_2 critical point technique (CPD 030 Balzers, Liechtenstein), fixed on aluminum stubs, covered with gold film and examined in a JEOL JSM-6060 microscope. Vancomycin (1 µg/mL) was used as antibiotic control.

2.8. In vivo assays using Galleria mellonella

2.8.1. Survival assay

Galleria mellonella larvae (200 mg) were randomly distributed in groups (*n* = 10/group) and infected with 10 µL of *S. aureus* UFPEDA-02 (ATCC-6538) suspension (1×10^5 CFU/larvae) injected into the last left proleg. After 2 h incubation at 37 °C, larvae received a single dose of 10 μL of *S. coronata* essential oil solutions at MIC or 2×MIC (that resulted in doses of 15.6 mg/kg or 31.2 mg/kg, respectively) and the plates were re-incubated at 37 ºC. Larvae infected with *S. aureus* and inoculated with vehicle (PBS) were used as positive control, while uninfected larvae also treated with vehicle were taken as negative control. Mortality rates of each group were observed daily during 5 days.

2.8.2. Early melanization assay

The effect of SCEO in the production of melanin induced by *S. aureus* infection was measured as previously described by Scorzoni et al. [34] with modifications. The larvae (*n*=10/group) were infected with *S. aureus* (1×10^6 CFU/larva) and immediately treated with SCEO (15.6 or 31.2 mg/kg). Larvae infected and treated with vehicle (PBS) were used as positive control while larvae inoculated only with vehicle composed the negative group. After 1 h and 3 h of incubation, the hemolymph of four larvae from each group was collected by cutting them with a scalpel blade through the cephalocaudal direction and squeezing. The obtained hemolymph was diluted in cold PBS and the melanin production was detected by measuring the absorbance at 405 nm.

2.8.3. Bacterial load in hemolymph

To evaluate the effect of SCEO in bacterial load in hemolymph, the larvae (*n* = 10/group) were infected with *S. aureus* (1×10^5 CFU) and treated with essential oil (31.2 mg/kg) as described in section 2.8.1. The hemolymph of five larvae was collected daily for 3 days, serially diluted in PBS and 4 µL of each dilution was plated on MHA. After incubation for 24 h at 37 ^{o}C, the number of CFU/mL was determined.

2.9. Statistical analysis

All assays were performed in triplicate in at least two independent experiments. Statistical analyses were performed by one-way analysis of variance (ANOVA). All analyses were carried out using GraphPrism, version 7. Differences were considered significant at $p < 0.05$. Differences in the survival of *G. mellonella* larvae were determined using the Kaplan-Meier method and log-rank test was used to compare survival curves.

3. Results and discussion

3.1. Chemical composition of SCEO

The hydrodistillation of *S. coronata* seeds allowed to obtain the essential oil with yield of 0.41±0.1%. The SCEO components detected by GC/MS and GC/FID are presented in Table 1. A total of 11 volatile constituents were identified, corresponding to 98.63% of the total oil, being most of them fatty acids (89.79%) and sesquiterpenes (8.5%). The most abundant components were octanoic acid, dodecanoic acid, decanoic acid and γ-eudesmol. Previous studies have reported that *S. coronata* oils are dominated by free carboxylic acids, accounting for approximately 80% of the total composition, and octanoic acid has been reported as the major volatile component of *S. coronata* oil [15, 16, 19].

3.2. Phenotypic characteristics of S. aureus isolates

The twenty *S. aureus* clinical isolates were from several sources such as catheter tip, purulent exudate, bone fragment, surgical wound and human lesions (Table 2). The results showed that 13 strains were resistant to oxacillin and classified as MRSA [35]. In fact, 11 MRSA clinical isolates were multidrug resistant as they showed resistance to at least 3 antibiotics while 1 MSSA was a multidrug resistant strain (Table 3). Microtiterplate assay revealed that 13 clinical isolates were strong biofilm producers, 6 strains were moderate biofilm producers, while one strain was a weak biofilm producer (Table 2).

3.3. SCEO is a bactericide agent against S. aureus

SCEO showed antimicrobial efficacy against all selected isolates of *S. aureus*, including those with biofilm-forming and multiple drug resistance phenotypes. The MIC values for the oil ranged from 156 μg/mL to 625 μg/mL (Table 3). The MBC values were equal to or 2-fold higher than each respective MIC, ranging from 312 to 1250 μg/mL, indicating the bactericidal effect of the oil. The MIC_{50} and MIC_{90} corresponded to 312 and 625 μg/mL, respectively. Essential oils from plants such *Caryophyllus aromaticus*, *Cinnamomum zeylanicum*, *Eugenia uniflora*, *Rosmarinus officinalis*, *Vernonia polyanthes*, and *Baccharis dracunculifolia* have been shown to be effective against clinical isolates of *S. aureus*, with MIC ranging from 0.25 to 56 mg/mL for MRSA and 0.25 to 50.8 mg/mL for MSSA [36].

The main constituents of SCEO are medium chain fatty acids, which have previously been identified as bioactive components against bacteria and yeasts, tending to be more active against gram-positive bacteria than gram-negative [37, 38]. For example, the octanoic acid, the major component of SCEO, has antibacterial properties against a range of gram-positive and gram-negative pathogens; dodecanoic and decanoic acids have been also reported as antimicrobial agents [39, 40].

3.4. SCEO effects viability of eradicates S. aureus biofilm

The effect of SCEO on the biomass and viability of preformed biofilm was evaluated using the strong biofilm producer *S. aureus* strain UFPEDA 02 (ATCC-6538). SCEO showed a slightly effect on biofilm matrix; significant reduction was observed only with 312 and 624 µg/mL concentrations (Figure 1). These findings were similar to vancomycin results, used as antibiotic control. On the other hand, SCEO was able to significantly decrease cell viability inside of the biofilm structure at all tested concentrations. Bacterial cell viability decreased more than 50% when the biofilm was submitted to the lowest concentration (156 µg/mL, corresponding to 0.5×MIC), while minimal viability was detected when the biofilms were exposed to the highest concentrations (624 and 1,248 µg/mL, corresponding to 2×MIC and 4×MIC 2) (Figure 1). Although the cell viability was strongly reduced in the treatment at 1248 µg/mL, there was no significant reduction in biofilm biomass, which can be due to a defensive response of the bacterial cells to this high oil concentration before they became inviable. Vancomycin showed low effect against the bacteria within the biofilm, confirming that planktonic bacterial susceptibility to antibiotics may not correspond to a good prediction for bacteria in biofilm lifestyle. This may represent a key point in the failure of antimicrobial treatment in the clinical routine as well as in the evaluation and development of new antimicrobial agents [41, 42].

The SEM analysis revealed untreated *S. aureus* biofilm as aggregates composed by cells with preserved structure (Figure 2A). No remarkable alterations were observed in biofilm treated with vancomycin (Figure 2B). SCEO treatments with MIC, 312 µg/mL

(Figure 2C), and supra-MIC, 624 μg/mL (Figure 2D) concentrations decreased the number of live cells in biofilms and led to loss of the roughness in the multiple layers of the three-dimensional structure of bacterial biofilm. SCEO at 312 μg/mL caused alteration in the cellular structure of *S. aureus* (Figure 2C), which may be related to the bactericidal action of the oil. Another effect induced by SCEO in *S. aureus* biofilms was the overproduction of exopolymeric matrix (Figure 2D), which can be a protective mechanism against the aggression caused by the treatment [43–45]. This datum corroborates with those reported in Figure 1. The maintenance of the three-dimensional matrix architecture (with dense areas, pores and channels) is crucial to determine the way of life in biofilm due to its influence on factors such as diffusion of nutrients, oxygen, residual products, and motility [46, 47]. The biofilm eradication ability of the major components of SCEO was already reported. Hogan et al. [48] demonstrated that application of ML:8, an emulsion based on octanoic acid, reduced *S. aureus* biofilm viability in more than 97% after 24 h treatment *in vitro*. Hess et al. [49] showed that dodecanoic acid was also able to reduce the viability of biofilm cells of *S. aureus*; however, it did not reduce the biofilm biomass.

3.5. SCEO reduces the deleterious effects of S. aureus infection in G. mellonella

Based on the MIC values, we selected two concentrations of SCEO to evaluate its antimicrobial action using *G. mellonella* larvae. The inoculation of SCEO at 15.6 mg/kg or 31.2 mg/kg did not change the survival rate of *G. mellonella* larvae. In addition, *G. mellonella* larvae exposed to SCEO developed the pupal stage in the same time period than untreated larvae. These data show that SCEO showed no toxicity to this insect.

The survival rate of *G. mellonella* larvae was reduced by infection with *S. aureus* UFPEDA-02, resulting in the death of all larvae in 3 days. This effect was inhibited when the larvae was treated with a single SCEO dose of 31.2 mg/kg, which resulted in survival rate of 60% in 4 days after infection. The mortality rate at dose of 15.6 mg/kg was not significantly different to that of the untreated group (Figure 3A). Aiming to investigate if the mortality rate was related to antibacterial activity of SCEO, the bacterial survival in hemolymph was evaluated. The hemolymph of larvae infected with *S. aureus* exhibited increased levels of bacterial load, approximately, 6, 8, and 9 log CFU/mL in 1^{st}, 2^{nd}, and 3^{rd} days post-infection, respectively. These values were reduced in treatment with SCEO at 31.2 mg/kg to 4, 5, and 5 log CFU/mL 1, 2, and 3 days post-infection, respectively (Figure 3B).

We also analyzed the effect of SCEO at 31.2 mg/kg in melanin production, employing a model of acute infection by inoculating the larvae with a high-density inoculum. Melanogenesis is an essential component of *G. mellonella* immune response against microbial infection [50]. However, the overproduction of this pigment has been associated to death induced by microorganisms [51]. The melanin in hemolymph significantly increased after 1 and 3 h of infection with *S. aureus* but SCEO was able to reduce larvae melanization induced by *S. aureus* infection in both periods (Figure 3C). These results corroborate with the benefic effects of this oil in infected larvae.

4. Conclusion

This work demonstrated that SCEO is an antibacterial agent against *S. aureus* strains with different resistance phenotypes. In addition, the oil was able to disturb biofilm formed by a strong biofilm producer isolate, and this antibiofilm activity was probably associated to the decrease of viability of cells inside the biofilm. *In vivo* antibacterial activity of SCEO against *S. aureus* improved survival of *G. mellonella* larvae and this fact indicates SCEO as a potential candidate to drug development for treatment of *S. aureus* infections.

Acknowledgments

The authors express their gratitude to the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) for research grants (446902/2014-4) and fellowships (MTSC, AJM, THN and PMGP). They are also grateful to the *Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco* (FACEPE; APQ-0108-2.08/14; APQ-0661-2.08/15), *Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul* (FAPERGS; PRONEM 16/2551-0000244-4); *Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão* (FAPEMA; COOPI-02860/16); and the *Coordenacão de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES; AUXPE 1454/2013) for financial support. KAK thanks the support of the International Network Programme, Danish Agency for Science, Technology and Innovation (6144-00103B). BSS would like to thank FACEPE for graduate scholarship (IBPG-0775-2.08/13). The authors are also grateful to technical assistance from the CMM/UFRGS for the microscopies (SEM).

References

[1] C.L. Ventola, The antibiotic resistance crisis: part 1: causes and threats. P T 40 (2015) 277-283.

[2] A.E. Paharik, A.R. Horswill, The staphylococcal biofilm: adhesins, regulation, and host response. Microbiol Spectr 4 (2016) doi:10.1128/microbiolspec

[3] F.E. Guerra, T.R. Borgogna, D.M. Patel, E.W. Sward, J.M. Voyich, Epic immune battles of history: neutrophils vs. *Staphylococcus aureus*, Front. Cell Infect. Microbiol. 7 (2017), 286.

[4] G. Pietrocola, G. Nobile, S. Rindi, P. Speziale, *Staphylococcus aureus* manipulates innate immunity through own and host-expressed proteases, Front. Cell Infect. Microbiol. 7 (2017) 166.

[5] R.A. Cosimi, N. Beik, D.W. Kubiak, J.A. Johnson, Ceftaroline for severe methicillin-resistant *Staphylococcus aureus* infections: a systematic review. Open Forum Infect. Dis. 4 (2017), ofx084.

[6] W.J.B. van Wamel, *Staphylococcus aureus* infections, some second thoughts, Curr. Opin. Infect. Dis. 30 (2017) 303-308.

[7] J. Haaber, J.R. Penades, H. Ingmer, Transfer of antibiotic resistance in *Staphylococcus aureus*, Trends Microbiol. 25 (2017) 893-905.

[8] A.M.S. Figueiredo, F.A. Ferreira, C.O. Beltrame, M.F. Cortes, The role of biofilms in persistent infections and factors involved in *ica*-independent biofilm development and gene regulation in *Staphylococcus aureus*, Crit. Rev. Microbiol. 43 (2017) 602-620.

[9] D.E. Moormeier, K.W. Bayles, *Staphylococcus aureus* biofilm: a complex developmental organism, Mol. Microbiol. 104 (2017) 365-376.

[10] H. Mccarthy, J.K. Rudkin, N.S. Black, L. Gallagher, E. O'neill, J.P. O'gara, Methicillin resistance and the biofilm phenotype in *Staphylococcus aureus*, Front Cell Infect Microbiol 5 (2015) 1.

[11] B.S. Santos, L.C.N. Silva, T.D. Silva, J.F. Rodrigues, M.A. Grisotto, M.T.S. Correia, T.H. Napoleão, M.V. Silva, P.M.G. Paiva, Application of omics technologies for evaluation of antibacterial mechanisms of action of plant-derived products. Front Microbiol 7 (2016) 1466.

[12] M.K. Swamy, M.S. Akhtar, U.R. Sinniah, Antimicrobial properties of plant essential oils against human pathogens and their mode of action: an updated review. Evid. Based Complement. Alternat. Med. 2016 (2016) 3012462.

[13] Silva, L.C.N., Silva, M.V., Correia, M.T.S., Editorial: New frontiers in the search of antimicrobials agents from natural products, Front Microbiol 8 (2017) 210.

[14] M.T. Islam, A.M. Mata, R.P. Aguiar, M.F. Paz, M.V. Alencar, P.M. Ferreira, A.A.C. Melo-Cavalcante, Therapeutic potential of essential oils focusing on diterpenes, Phytother Res 30 (2016) 1420-1444.

[15] S. Belviso, D. Ghirardello, M. Giordano, G.S. Ribeiro, J.S. Alves, S. Parodi, S. Risso, G. Zeppa, Phenolic composition, antioxidant capacity and volatile compounds of licuri (*Syagrus coronata* (Martius) Beccari) fruits as affected by the traditional roasting process, Food Res. Int. 51 (2013) 39-45.

[16] C.M.A. Bessa, R.S.D. Nascimento, R.C.C. Alves, J.M. Anselmo, A.P.S. Silva, A.G. Silva, V.L.M. Lima, J.F. Tavares, L.C.N. Silva, M.V. Silva, M.T.S. Correia, *Syagrus coronata* seed oils have antimicrobial action against multidrug-resistant *Staphylococcus aureus*, J. Med. Plants Res. 10 (2016) 310-317.

[17] E. Toensmeier, The carbon farming solution: a global toolkit of perennial crops and regenerative agriculture practices for climate change mitigation and food security. Chelsea Green Publishing, Vermont (2016).

[18] W.M.D. Andrade, M.A. Ramos, W.M.S. Souto, J.S. Bento-Silva, U.P.D. Albuquerque, E.D.L. Araújo, Knowledge uses and practices of the licuri palm (*Syagrus coronata* (Mart.) Becc.) around protected areas in northeastern Brazil holding the endangered species lear's macaw (*Anodorhynchus leari*), Trop. Conserv. Sci. 8 (2015) 893-911.

[19] L.M.M. Santos, J.S. Nascimento, M.G. Santos, N.B. Marriel, P.C. Bezerra-Silva, S.K.L. Rocha, A.G. Silva, M.T.S. Correia, P.M.G. Paiva, G.F. Martins, M.V. Silva, T.H. Napoleão, Fatty acid-rich volatile oil from *Syagrus coronata* seeds has larvicidal and oviposition-deterrent activities against *Aedes aegypti*, Physiol. Mol. Plant Pathol. 100 (2017) 35-40.

[20] L.B. Leal. G.D. Sousa, K.B. Seixas, P.H.N.D. Souza, D.P.D. Santana, Determination of the critical hydrophile-lipophile balance of licuri oil from *Syagrus coronata*: Application for topical emulsions and evaluation of its hydrating function, Braz. J. Pharm. Sci. 49 (2013) 167-173.

[21] T.A.F. Ferro, J.M.M. Araujo, B.L.S. Pinto, J.S. Santos, E.B. Souza, B.L.R. Silva, V.L.P. Colares, T.M.G. Novais, C.M.B. Filho, C. Struve, J.B. Calixto, V. Monteiro-Neto, L.C.N. Silva, E.S. Fernandes, Cinnamaldehyde inhibits *Staphylococcus aureus* virulence factors and protects against infection in a *Galleria mellonella* model, Front Microbiol 7 (2016) 2052.

[22] R. Jønsson, C. Struve, H. Jenssen, K.A. Krogfelt, The wax moth *Galleria mellonella* as a novel model system to study enteroaggregative *Escherichia coli* pathogenesis, Virulence 8 (2017) 1894-1899.

[23] K.M.T. Astvad, J. Meletiadis, S. Whalley, M.C. Arendrup, Fluconazole pharmacokinetics in the *Galleria mellonella* larvae and performance evaluation of a bioassay compared to LC-MS/MS for haemolymph specimens, Antimicrob Agents Chemother. 61 (2017) e00895-17.

[24] L.C.M. Lima, D.M.A.F. Navarro, L.P. Souza-Santos, Effect of diet on the fatty acid composition of the copepod *Tisbe biminiensis*, J. Crust. Biol. 33 (2013) 372-381.

[25] E.S. Autran, I.A. Neves, C.S.B. Silva, G.K.N. Santos, C.A.G. Câmara, D.M.A.F., Chemical composition, oviposition deterrent and larvicidal activities against *Aedes aegypti* of essential oils from *Piper marginatum* Jacq. (Piperaceae), Bioresour Technol 100 (2009), 2284-2288.

[26] H. van Den Dool, P.D. Kratz, A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography, J Chromatogr 11 (1963) 463-471.

[27] R.P. Adams, Identification of essential oil components by gas chromatography/mass spectroscopy, Allured Publishing, Carol Stream, USA.

[28] A.W. Bauer, M.D.K. Kirby, J.C. Sherries, M. Truck, Antibiotic susceptibility testing by a standardized single disk method, Am. J. Clin. Pathol. 45 (1966) 493-496.

[29] P.H. Krumperman, Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods, Appl. Environ. Microbiol. 46 (1983) 165-170.

[30] S. Stepanovic, D. Vukovic, V. Hola, G. Di Bonaventura, S. Djukic, I. Cirkovic, F. Ruzicka, Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci, APMIS 115 (2007) 891-899.

[31] Clinical and Laboratory Standards Institute, Performance standards for antimicrobial susceptibility testing; 24th informational supplement. CLSI document M100-S24 (2014).

[32] K.R. Zimmer, A.J. Macedo, R.B. Giordani, J.M. Conceicao, G.G. Nicastro, A.L. Boechat, R.L. Baldini, W.R. Abraham, C. Termignoni, A steroidal molecule present in the egg wax of the tick *Rhipicephalus* (*Boophilus*) *microplus* inhibits bacterial biofilms. Environ. Microbiol. 15 (2013) 2008-2018.

[33] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays, J. Immunol. Meth. 65 (1983) 55–63.

[34] L. Scorzoni, M.P. Lucas, A.C. Mesa-Arango, A.M. Fusco-Almeida, E. Lozano, M. Cuenca-Estrella, M.J. Mendes-Giannini, O. Zaragoza, Antifungal efficacy during *Candida krusei* infection in non-conventional models correlates with the yeast in vitro susceptibility profile, PLoS One 8 (2013) e60047.

[35] V.A. Kumar, K. Steffy, M. Chatterjee, M. Sugumar, K.R. Dinesh, A. Manoharan, S. Karim, R. Biswas, Detection of oxacillin-susceptible mecA-positive *Staphylococcus aureus* isolates by use of chromogenic medium MRSA ID, J. Clin. Microbiol. 51 (2013) 318-319.

[36] L.N. Barbosa, I.S. Probst, B.F.M.T. Andrade, F.C.B. Alves, M. Albano, L.M.C. M.L.R.S. Cunha, J.T. Doyama, V.L.M. Rall, A.F. Júnior, A. In vitro antibacterial and chemical properties of essential oils including native plants from Brazil against pathogenic and resistant bacteria, J. Oleo Sci. 64 (2015), 289-298.

[37] A.P. Desbois, V.J. Smith, Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential, Appl. Microbiol. Biotechnol. 85 (2010) 1629-1642.

[38] E.M.S. Lillebæk, S.L. Nielsen, R.S. Thomasen, N.J. Faergeman, B.H. Kallipolitis, Antimicrobial medium- and long-chain free fatty acids prevent PrfA-dependent activation of virulence genes in *Listeria monocytogenes*, Res. Microbiol. 168 (2017) 547-557.

[39] R. Hulankova, G. Borilova, I. Steinhauserova, Combined antimicrobial effect of oregano essential oil and caprylic acid in minced beef. Meat Sci 95 (2013) 190-194.

[40] S.A. Kim, M.S. Rhee, Synergistic antimicrobial activity of caprylic acid in combination with citric acid against both *Escherichia coli* O157:H7 and indigenous microflora in carrot juice, Food Microbiol. 49 (2015), 166-172.

[41] J. Claessens, M. Roriz, R. Merckx, P. Baatsen, L. Van Mellaert, J. Van Eldere, Inefficacy of vancomycin and teicoplanin in eradicating and killing *Staphylococcus epidermidis* biofilms in vitro, Int. J. Antimicrob. Agents 45 (2015) 368-375.

[42] B. Ozturk, N. Gunay, B.M. Ertugrul, S. Sakarya, Effects of vancomycin, daptomycin, and tigecycline on coagulase-negative staphylococcus biofilm and bacterial viability within biofilm: an in vitro biofilm model, Can. J. Microbiol. 62 (2016) 735-743.

[43] D.S. Trentin, R.B. Giordani, K.R. Zimmer, A.G. Silva, M.V. Silva, M.T.S. Correia, I.J. Baumvol, A.J. Macedo, Potential of medicinal plants from the Brazilian semi-arid region (Caatinga) against *Staphylococcus epidermidis* planktonic and biofilm lifestyles, J. Ethnopharmacol. 137 (2011) 327-335.

[44] Z. Sanchez, A. Tani, K. Kimbara, Extensive reduction of cell viability and enhanced matrix production in *Pseudomonas aeruginosa* PAO1 flow biofilms treated with a D-amino acid mixture, Appl. Environ. Microbiol. 79 (2013) 1396 –1399.

[45] B. Kundukad, M. Schussman, K. Yang, T. Seviour, L. Yang, S.A. Rice, S. Kjelleberg, P.S. Doyle, Mechanistic action of weak acid drugs on biofilms, Sci. Rep. 7 (2017) 4783.

[46] H.C. Flemming, J. Wingender, The biofilm matrix, Nat. Rev. Microbiol. 8 (2010), 623-633.

[47] H.C. Flemming, J. Wingender, U. Szewzyk, P. Steinberg, S.A. Rice, S. Kjelleberg, Biofilms: an emergent form of bacterial life, Nat Rev Microbiol 14 (2016) 563-575.

[48] S. Hogan, M. Zapotoczna, N.T. Stevens, H. Humphreys, J.P. O'gara, E. O'neill, Eradication of *Staphylococcus aureus* catheter-related biofilm infections using ML:8 and Citrox, Antimicrob. Agents Chemother. 60 (2016) 5968-5975.

[49] D.J. Hess, M.J. Henry-Stanley, C.L. Wells, The natural surfactant glycerol monolaurate significantly reduces development of *Staphylococcus aureus* and *Enterococcus faecalis* biofilms, Surg. Infect. 16 (2015) 538-542.

[50] A. Zdybicka-Barabas, P. Mak, T. Jakubowicz, M. Cytrynska, Lysozyme and defense peptides as suppressors of phenoloxidase activity in *Galleria mellonella*, Arch. Insect Biochem. Physiol. 87 (2014) 1-12.

[51] H. Ciesielczuk, J. Betts, L. Phee, M. Doumith, R. Hope, N. Woodford, D.W. Wareham, Comparative virulence of urinary and bloodstream isolates of extra-intestinal pathogenic *Escherichia coli* in a *Galleria mellonella* model, Virulence 6 (2015) 145-151.

Figure captions

Figure 1. Effect of the *Syagrus coronata* essential oil (SCEO) on biomass and cell viability in *S. aureus* UFPEDA-02 biofilm. Biomass was quantified using the microtiterplate method (OD 570 nm) and viability was determined by MTT assay (OD 540 nm). (*) $p<0.05$; (**) $p<0.01$.

Figure 2. SEM images of biofilms formed by *S. aureus* UFPEDA-02. (A) Untreated biofilm. (B) Biofilm treated with vancomycin at 1 µg/mL. (C) Biofilm treated with *Syagrus coronata* essential oil (SCEO) at 312 µg/mL. Arrows point cells with altered structure. (D) Biofilm treated with SCEO at 624 µg/mL. The asterisks indicate overproduction of exopolymeric matrix.

Figure 3. Effects of SCEO on *Galleria mellonella* larvae infected with *S. aureus* UFPEDA-02. (A) Survival curves of uninfected insects treated with PBS as well as infected insects treated with PBS (control) or SCEO at 15.6 and 31.2 mg/kg. (B) Bacterial load and (C) melanization in larvae uninfected treated with PBS as well as insects treated with PBS (control) or SCEO at 31.2 mg/kg. (*) $p<0.05$ (**) $p<0.01$.

Table 1. Constituents of *Syagrus coronata* seed essential oil (SCEO).

Nº	Compound[a]	RI		Content (as % of total oil)
		Determined[b]	Literature[c]	
1	Octanoic acid	1195	1167	46.77 ± 1.85
2	Decanoic acid	1378	1364	20.93 ± 0.29
3	*trans*-Caryophyllene	1421	1417	0.41 ± 0.05
4	Viridiflorene	1497	1496	0.53 ± 0.09
5	δ-Cadinene	1525	1522	0.44 ± 0.06
6	Dodecanoic acid	1573	1565	22.09 ± 3.51
7	Caryophyllene oxide	1586	1582	0.61 ± 0.23
8	Ethyl dodecanoate	1595	1594	0.34 ± 0.06
9	γ-Eudesmol	1634	1630	4.26 ± 0.41
10	β-Eudesmol	1653	1649	0.41 ± 0.04
11	α-Eudesmol	1656	1652	1.84 ± 0.61
	Total			**98.63**

[a] Constituents listed in order of elution on a non-polar DB-5 column; [b]Retention indexes (RI) calculated from retention times in relation a series of C_8-C_{30} *n*-alkanes on a 30 m DB-5 capillary column; [c]Values taken from Adams (2007).

Isolate	Source	Phenotypic evaluation	Crystal violet assay

Table 2. Isolation source, phenotypic evaluation and biofilm formation ability of *S. aureus* isolates used in this study.

		Colony color	Colony consistency	OD_{570}	Biofilm formation
UFPEDA-02 (ATCC-6538)	Human lesion	Almost black	Dry	1.47±0.12	+++
UFPEDA-659	Catheter tip	Red	Crystalline	0.59±0.13	++
UFPEDA-662	Catheter tip	Almost black	Rough	1.22±0.08	+++
UFPEDA-670	Catheter tip	Red	Crystalline	0.52±0.03	++
UFPEDA-671	Bone Fragment	Almost black	Dry e Rough	1.03±0.15	+++
UFPEDA-672	Bone Fragment	Almost black	Rough	1.14±0.08	+++
UFPEDA-674	Purulent exudate	Bordeaux red	Crystalline	0.62±0.09	++
UFPEDA-679	Surgical wound	Black	Rough	0.77±0.07	++
UFPEDA-683	Purulent exudate	Almost black	Rough	1.06±0.19	+++
UFPEDA-689	Purulent exudate	Black	Rough	1.22±0.11	+++
UFPEDA-691	Catheter tip	Red	Rough	0.45±0.06	++
UFPEDA-699	Catheter tip	Red	Crystalline	1.01±0.15	+++
UFPEDA-700	Diabetic foot ulcer	Bordeaux red	Crystalline	1.01±0.22	+++
UFPEDA-705	Surgical wound	Black	Rough	1.49±0.18	+++
UFPEDA-709	Purulent exudate	Red	Crystalline	1.31±0.09	+++
UFPEDA-718	Tracheal secretion	Red	Crystalline	0.38±0.05	+
UFPEDA-726	Nasal secretion	Red	Crystalline	1.18±0.12	+++
UFPEDA-731	Surgical wound	Almost black	Rough	0.58±0.05	++
UFPEDA-733	Bone Fragment	Bordeaux red	Crystalline	1.28±0.16	+++
UFPEDA-802	Nasal secretion	Red	Dry	1.14±0.2	+++

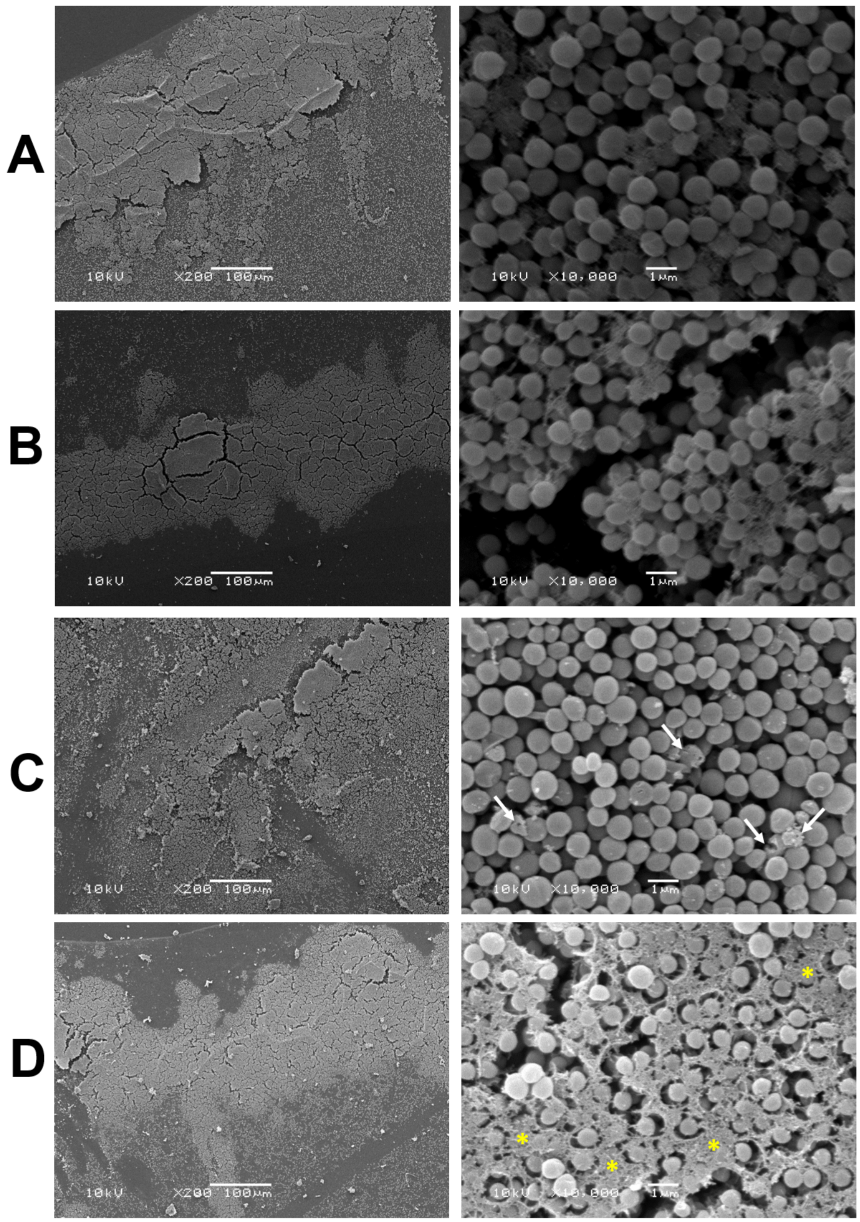
(+++) Strong biofilm forming strain. (++) Moderate biofilm forming strain. (+) Weak biofilm forming strain.

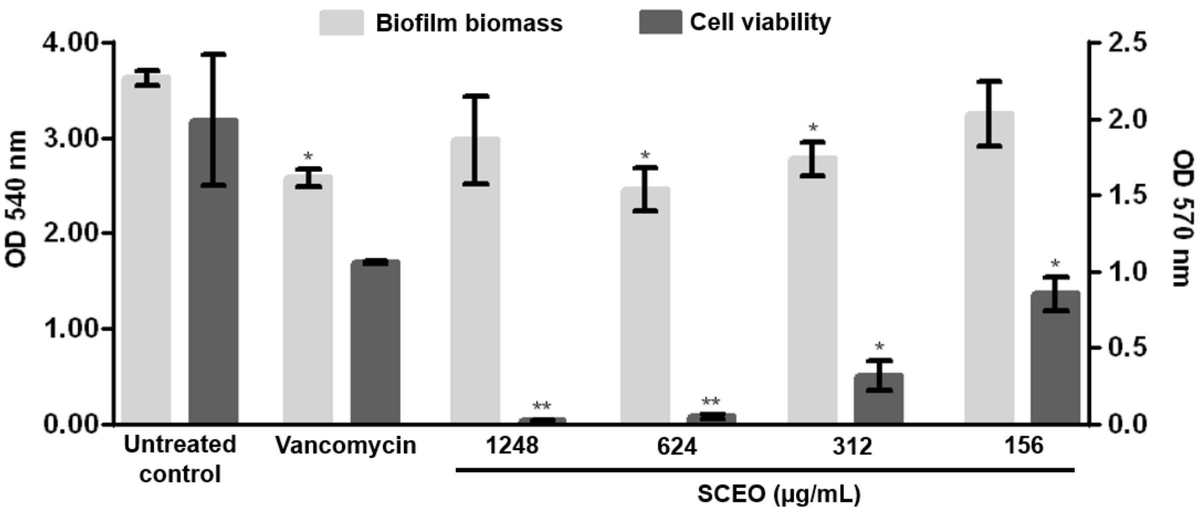
Table 3. Antibiotic resistance profile of *S. aureus* isolates and antibacterial activity of *Syagrus coronata* essential oil (SCEO).

Clinical isolate	Susceptibility profile	MAR	SCEO activity	
			MIC(µg/mL)	MBC
UFPEDA-02	Susceptible	0	312	312
UFPEDA-659	CFO,OXA, NAL	0.15	312	312
UFPEDA-662	AMP, CFO, OXA, NAL	0.2	625	625
UFPEDA-670	AMP, CFO, OXA, NAL, CIP, CLI, TRI	0.35	312	625
UFPEDA-671	AMP, CFO, OXA, NAL, CIP, AMI, GEN, CLI, CLO, TET, TRI	0.55	312	312
UFPEDA-672	AMP, CFO, OXA,NAL, CIP, NIT, CLI, TRI	0.4	156	312
UFPEDA-674	AMP, NAL, CLI, TET	0.2	312	625
UFPEDA-679	AMP, CFO, OXA, CFL, CFZ, NAL, VAN, AMI, CLI	0.45	625	625
UFPEDA-683	AMP, OXA, CFL, CFO, CFZ, CPM, CRX, CTX, NAL, CIP, VAN, AMI, GEN, CLI, CLO, TRI	0.8	625	1250
UFPEDA-689	AMP, CFZ, NAL, GEN, CLI, CLO, TET, TRI	0.4	625	625
UFPEDA-691	NAL, CIP, CLO	0.15	156	312
UFPEDA-699	AMP, NAL, CLI, CLO	0.2	156	312
UFPEDA-700	AMP, CFO, OXA, CIP, TET	0.25	312	156
UFPEDA-705	AMP, OXA, CFL, CFO, CPM, CRX, NAL, NIT, GEN	0.45	312	312
UFPEDA-709	AMP, CFO, OXA, NAL, CLI, TET	0.3	625	625
UFPEDA-718	AMP, NAL, CIP	0.15	312	312
UFPEDA-726	AMP, CFO, OXA, CIP, GEN, CLO, TRI	0.35	312	312
UFPEDA-731	AMP, CFO, OXA, CFL, CFO, CRX, NAL, CIP, GEN, CLI, CLO, TRI	0.6	312	312
UFPEDA-733	AMP, NAL, CIP, CLO	0.2	625	625
UFPEDA-802	AMP, OXA, CFL, CFO, CFZ, CPM, CRX, CTX, NAL, CIP, AMI , GEN , CLI , CLO, TET,	0.8	625	625

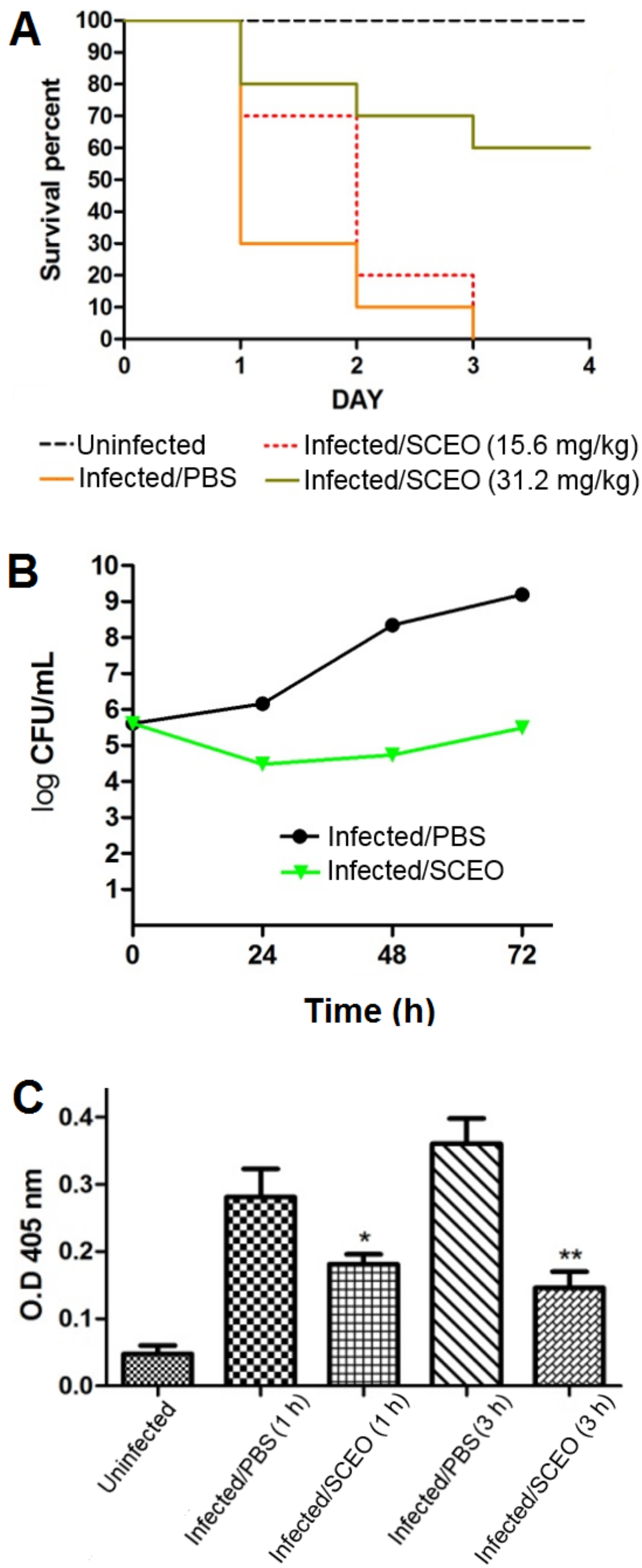
AMP: ampicillin. OXA: oxacillin. CFL: cephalothin. CFZ: cefazolin. CPM: cefepime. CFO: cefoxitin, CTX: cefotaxime. CRX: cefuroxime. IMI: imipenem. MER: meropenem. NAL: nalidixic acid. CIP: ciprofloxacin. NIT: nitrofurantoin. AMI: amikacin. GEN: gentamicin. VAN: vancomycin. CLI: clindamycin. CLO: chloramphenicol. TET: tetracycline. TRI: trimethoprim. MAR: multiple antibiotic resistance index. MIC:

minimum inhibitory concentration. MBC: minimum bactericidal concentration. The MIC_{50} and MIC_{90} of SCEO were 312 and 625 μg/mL, respectively.


A
10kV ×200 100μm
10kV ×10,000 1μm
B
10kV ×200 100μm
10kV ×10,000 1μm
C
10kV ×200 100μm
10kV ×10,000 1μm
D
10kV ×200 100μm
10kV ×10,000 1μm



Biofilm biomass
Cell viability
OD 540 nm
OD 570 nm
4.00
3.00
2.00
1.00
0.00
2.5
2.0
1.5
1.0
0.5
0.0
Untreated control
Vancomycin
1248
624
312
156
SCEO (µg/mL)


A
Survival percent
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
DAY
Uninfected
Infected/PBS
Infected/SCEO (15.6 mg/kg)
Infected/SCEO (31.2 mg/kg)
B
log CFU/mL
10
9
8
7
6
5
4
3
2
1
0
24
48
72
Time (h)
Infected/PBS
Infected/SCEO
C
O.D 405 nm
0.4
0.3
0.2
0.1
0.0
*
**
Uninfected
Infected/PBS (1 h)
Infected/SCEO (1 h)
Infected/PBS (3 h)
Infected/SCEO (3 h)

Highlights

- Essential oil extracted from *Syagrus coronata* seeds (SCEO) was obtained.
- SCEO showed bactericidal activity (MBC from 312 to 1250 µg/mL) against *S. aureus.*
- SCEO decreased cell viability in pre-formed biofilms of *S. aureus* isolate.
- SCEO improved the survival of *G. mellonela* larvae inoculated with *S. aureus.*