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Talinum paniculatum leaves with in vitro antimicrobial activity against reference and clinical strains of *Staphylococcus aureus* interfere with oxacillin action

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Summary

Propose: We evaluated the antibacterial potential of the crude leaf extract (CLE) and fractions hexane (HX) and ethyl acetate (EtOAc) from *Talinum paniculatum* alone and in association with oxacillin (OXA) against OXA-resistant *Staphylococcus aureus* (ORSA, environment isolates) and OXA-sensitive *S. aureus* (OSSA, ATCC 25923). Furthermore, toxicity tests were performed. **Methods:** The antibacterial activity was evaluated through checkerboard assay (broth microdilution) to establish the minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC). Toxicity test in mice was assessed. **Results:** The MIC values for the CLE and its fractions against ORSA and OSSA were in the order of HX (500 $\mu g\,ml^{-1}$) = EtOAc < CLE (4000 $\mu g\,ml^{-1}$). EtOAc and HX presented outstanding antibacterial activities against ORSA, and these fractions were bactericidal toward OSSA. Conversely, the associations between plant product (CLE, EtOAc, or HX) and OXA exhibited no synergistic effects. During these associations, there was an increase in OXA MICs anywhere from 2- to 4092-fold. The CLE presented absence of toxicity at a dose of 5 g kg^{-1} (*in vivo*). **Conclusion:** Although *T. paniculatum* be a good source

of bioactive compounds with antistaphylococcal potential, the researchers should be cautious, since its edible leaf may interfere with OXA therapy (mitigating OXA-induced growth inhibition or killing of *S. aureus* and enhancing *S. aureus* resistance).

Key words: Antimicrobial resistance, *Staphylococcus aureus*, reactive oxygen species, oxacillin, *Talinum paniculatum*.

Resumen

Las hojas de *Talinum paniculatum* con actividad antimicrobiana *in vitro* contra cepas de referencia y clínicas de *Staphylococcus aureus* interfieren con la acción de la oxacilina

Propósito: evaluamos el potencial antibacteriano del extracto de hoja en bruto (EHB) y las fracciones hexano (HX) y acetato de etilo (AcOEt) de *Talinum paniculatum* solo y en asociación con oxacilina (OXA) contra *Staphylococcus aureus* resistente a OXA (ORSA, ambientales) y *S. aureus* sensible a OXA (OSSA, ATCC 25923). Además, se realizaron pruebas de toxicidad. **Métodos:** la actividad antibacteriana se evaluó mediante microdilución en caldo para establecer las concentraciones inhibitorias mínimas (CIM) y bactericidas mínimas (CBM). Se evaluó la toxicidad en ratones. **Resultados:** los valores de CIM para el EHB y sus fracciones contra ORSA y OSSA fueron del orden de HX (500 $\mu g\,ml^{-1}$) = AcOEt < EHB (4000 $\mu g\,ml^{-1}$). AcOEt y HX presentaron actividades antibacterianas sobresalientes contra ORSA, y estas fracciones fueron bactericidas hacia OSSA. Por el contrario, las asociaciones entre el producto vegetal (EHB, AcOEt o HX) y OXA no mostraron efectos sinérgicos. Durante estas asociaciones, hubo un aumento en las CIM de OXA de 2 a 4092 veces. EHB no mostró toxicidad a una dosis de 5 $g\,kg^{-1}$. **Conclusión:** aunque *T. paniculatum* es una buena fuente de compuestos bioactivos con potencial antiestofilocócico, los investigadores deben ser cautelosos, ya que su hoja comestible puede interferir con la terapia con OXA (mitigando la inhibición del crecimiento inducida por OXA o la muerte de *S. aureus* y promoviendo resistencia bacteriana).

Palabras clave: Resistencia a los antimicrobianos, *Staphylococcus aureus*, oxacilina, *Talinum paniculatum*.

Introduction

Infectious diseases remain among the leading causes of morbidity and mortality in the world. Moreover, antimicrobial (AMR) and multidrug (MDR) resistance have risen to

alarming levels in *Staphylococcus aureus* (i.e., chiefly to penicillin and cross-resistance to other ß-lactams). From the global burden associated with AMR/MDR in microorganisms, most frequent are the threat to therapeutics, prolonged illness, decreased effectiveness, creating easy targets for immunocompromised conditions, high medical costs, and high mortality rates [1-4].

Indeed, the accelerating growth and global expansion of oxacillin (OXA) resistant *S. aureus* (ORSA) strains and hospital (HAI) or community (CAI) associated infections related to them are ultimately both a global public health problem and a critical problem in clinical settings where immunocompromised patients or those who have had surgical wounds are present. Resistance to current antibiotics has limited the therapeutic armory to treat *S. aureus* infections [6, 7].

In that way, approaches such as screening of plant products alone or in combination with antibiotics that can tackle AMR/MDR have been extensively reported [7-11]. Plants have been used in folk medicine since ancient times, and the chemical diversity and therapeutic potential are great reasons for the current interest taken in them [10]. *Talinum paniculatum* (Jacq.) Gaertner (Talinaceae family) is a plant widely spread throughout the world, commonly known as "Erva-gorda" and "Língua-de-vaca" in Brazil or Tu-ren-shen in Traditional Chinese Medicine [12]. Its green leafy is a non-conventional vegetable for human consumption [12]. Furthermore, several biological activities have been attributed to *T. paniculatum* and its bioactive compounds, including antibacterial and antifungal [7], estrogenic [13], antifertility [14], antinociceptive [15], and other effects: on uterine contractility and those related in folk medicine [12, 16, 17].

Over the last two decades, the underlying mechanism of action for the bactericidal ß-lactam antibiotics (including OXA) against a broader set of microorganisms has been shown to be overt dependent of reactive oxygen species (ROS) accumulation [18-20]. On this knife's edge, OXA-induced ROS-associated damage to proteins and/or DNA contribute to *S. aureus*' loss of viability, even when there is increased expression of systems of ROS detoxification (i.e. condition that characterizes the so-called oxidative stress) [21-23]. Indeed, β-lactams (including OXA) can generate oxidative stress-induced autolysis. Besides these factors, oxidative stress tolerance-induced antibiotic resistance in *S. aureus* biofilms with a maintenance of the steady-state levels of ROS in *S. aureus* cells, has been described [21-27].

Despite the controversy and opposing viewpoints over the mechanism of ROS accumulation behind the activity of antibiotics, OXA activity could be hampered by providing microbial cells with exogenous antioxidants, as attested in previous studies [20, 28-31]. Moreover, antioxidants could prime (priming) microbial cells to

counteract the oxidative stress induced by antibiotics [1, 22, 24, 32, 33]. Therefore, the elucidation of possible interactions between antioxidants-rich foods and antibiotic is important because of many nutrition products and medicines, including the leaves from *T. paniculatum* that are often used as a green leafy vegetable for human consumption, may occur during medical treatment and modify the action of the antibiotic hence this could have a great impact on clinical practice and patient outcomes [7, 12].

In this study, we evaluated the antistaphylococcal potential of the extract and fractions from the leaves of *T. paniculatum* alone or in association with the ß-lactam antibiotic OXA against ORSA and OXA-sensitive *S. aureus* (OSSA). Furthermore, a possible toxicity of *T. paniculatum* (*in vivo*) was checked.

Materials and methods

Chemicals

Oxacillin (OXA); Mueller Hinton broth (MHB); 7-Hydroxy-3H-phenoxazin-3-one 10-oxide (Resazurin) were purchased from Sigma-Aldrich, St. Louis, MO, USA. Nutriente Agar (Difco); Agar/broth Brain Heart Infusion (BHI, HiMedia Laboratories); Dimethyl sulfoxide (DMSO, Neon Comercial); Solutions: Sodium chloride 0.9% (145 mM NaCl); Phosphate-buffered saline (PBS, pH 7.4): NaCl (120 mM), KH_2PO_4 (1.7 mM), Na_2HPO_4 (8.3 mM) e KCl (5 mM). Other chemicals and solvents were analytical grade. The reagents were sterilized into an autoclave (whenever possible) or by filtration (Millipore Corporation, hydrophilic Durapore® PVDF, 0.22 μm, Ø 47 mm).

Ethics statement

This study did not involve any endangered or protected species and no specific permits were required for the described studies. Botanical material from *T. paniculatum* was collected in a particular area, with access permitted to researchers.

All animal experiments were carried out in strict accordance with the recommendations of the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health [NIH], Washington DC: The National Academy Press, 2011). The animals were gently conceded from a previous approved study by the ethics committee on the use of animals (CEUA) of the Federal University of Alfenas (protocol #490/2013).

Plant material and preparation of the crude leaf extract and its fractions

T. paniculatum leaves were collected in the mornings of June, near the town of Fama, MG state, Brazil (Geographic coordinates obtained from GPS observations: 21° 24' 53.4" S; 45° 52' 15.8'' W). The climate of this region is classified as humid temperate,

with a hot summer and a dry winter (type Cwa in the classification of Köppen). A voucher specimen was deposited in the UALF Herbarium at Federal University of Alfenas (UNIFAL-MG), after identification and taxonomic authentication by a botanical (Herbarium number 2338). The collected *T. paniculatum* leaves were weighed, arranged in thin layers, and dried in a circulating air oven at 40 °C for eleven days (250 h), aiming at a stable and constant weight to be reached. The final percentage of dry mass was 5.26%. The material underwent through a rough division followed by a spray obtained in a Wiley mill. After grinding, the size of the powder particles was measured according to WHO, as shown in table 1 [34]. The dried leaf powder (200 g) from *T. paniculatum* was percolated (with an alcohol to water ratio of 7:3) at 1.0 mL/min/kg. After fluid extraction, the fluid was placed in a rotary evaporator under reduced pressure and at a temperature of 45 °C. The extract was then lyophilized to completely remove water and obtain the dry extract, hereafter referred to as CLE (17.0% yield). To obtain the fractions, the dried extract was subjected to a liquid-liquid partition with hexane (1:1, v/v [4×]) and ethyl acetate (1:1, v/v [6×]), yielding hexane (HX), ethyl acetate (EtOAc), and a final residue termed the aqueous fraction. During this process, the solvents, hexane, and ethyl acetate, are removed. The CLE or fractions were solubilized in DMSO (which at used concentration does not present antimicrobial activity) and adjusted at used concentrations.

Table 1. Diameters of the mesh chosen to perform the powder grain size of leaves from *Talinum paniculatum*.

Mesh (n. º)	Average Diameter (µm)	Mass (g)	Percentage (%)
20	850	0.56	0.46
25	710	2.33	1.94
35	500	28.46	23.57
45	355	28.38	23.50
60	250	35.66	29.52
0	-	25.38	21.01
Total		120.77	

Activity against S. aureus

Strains

Reference strains of *S. aureus* were from the American Type Culture Collection (*Staphylococcus aureus subsp. aureus* [ATCC® 25923™ and 6538], Manassas, VA, USA). The two ORSA isolates were from clinical environment and identified as shown in

table 2. The classification of AMR in *S. aureus* was as following: OXA MIC ≤ 2 μg ml^{-1}= Sensitive and ≥ 4 μg ml^{-1}= resistant [35].

Table 2. Used microorganisms, clinical sources, identification, and code.

Microorganism	Code	Source	Identification methods
S. aureus	Sample G11.39	Environment isolates from air at a dental clinicz	Multiplex PCR
S. aureus	Sample G11.19	Environment isolates from air at a dental clinic	Multiplex PCR

Loci	Oligonucleotides: Primers F (forward) e R (reverse)	Size of the amplicons	Purpose/interpretation
mecA1	5' TGG CTA TCG TGT CAC AAT CG 3' (F) 5' CTG GAA CTT GTT GAG CAG AG 3' (R)	310 bp	Resistance profile: ORSA (+) OXA-sensitive *S. aureus* (-)
femA1	5' CTT ACT TAC TGG CTG TAC CTG 3' (F) 5' ATG TCG CTT GTT ATG TGC 3' (R)	686 bp	ORSA identification (level of OXA resistance): ORSA (+)
IS431[A]	5' AGG ATG TTA TCA CTG TAG CC 3' (F) 5' GAT GTA CAA TGA CAG TCA GG 3' (R)	444 bp	Class C1 *mec g*ene complex: ORSA: (+)

[A]Insertion sequence.

Genomic DNA: Clinical isolates of *S. aureus* was grown in Luria-Bertani medium (pH 7, Laboratory Bottles, Hamburg, Germany) for 16 hrs at 35 °C with continuous shaking (150 r.p.m). Then, bacteria were washed two times with Tris-buffered saline (TBS: 50 mM Tris HCl pH 8.0, 100 mM EDTA, 150 mM NaCl), followed by a centrifugation at 1500 × *g* (Centrifuge 5417R, Eppendorf do Brasil Ltda., São Paulo, SP, Brasil) for 5 min. The resulting *S. aureus* cells containing-pellet was incubated at 37 °C for 30 min and cells were lysed in 500 μL TBS containing SDS (1% m/v), 100 μg lysostaphin (Sigma Chemical Co., St. Louis, MO, USA) and 100 μg RNAse (Sigma Chemical Co., St. Louis, MO, USA) into Eppendorf Thermomixer® comfort (Eppendorf™, Hamburg, Germany). The final lysed *S. aureus* cells were added with 200 μg proteinase K (Sigma Chemical Co., St. Louis, MO, USA) and 200 μg lysozyme (Sigma Chemical Co., St. Louis, MO, USA). Next, after the addition of phenol:chloroform:isoamyl alcohol (25:24:1), a high-speed centrifugation (12000 ′ *g* for 10 min) was performed and the culture supernatants underwent a further centrifugation, and then resuspended in chloroform:isoamyl alcohol (24:1). Genomic DNA was pelleted by subsequent addition of ethanol at –20 °C for 30 min and centrifugation at 12000 × *g* for 10 min, dried at 30 °C for 90 min, and solubilized in 500 μL TE buffer (10 mM Tris, 1 mM EDTA, pH 8) and stored at –20 °C. **PCR multiplex assays** - PCR was performed using the following set up: 100 ng genomic DNA in assay buffer (20 mM Tris HCl pH 8.4, 50 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 100 pmol of each primer (described in **table 1**), and 1.5 U Taq DNA polymerase (Invitrogen™ Brasil Ltda.) were mixed and underwent to an initial program for DNA denaturation at 92 °C for 3 min, followed by 30 cycles at 92 °C for 1 min (denaturation), 56 °C for 1 min (annealing) and 72 °C for 1 min (extension). The final cycle was performed at 72 °C for 5 min for the final extension. **Amplicons patterns** - The PCR products in loading buffer (10% v/v glycerol, 0.083% m/v bromophenol blue, 0.083% m/v xylene cyanol in H_2O milliQ) were analyzed on a 2% agarose gel (prepared with TBE buffer 0.5′: 445 mM

trizma base, 445 mM boric acid, and 10 mM EDTA) and 0.5 μg mL^{-1} ethidium bromide) using horizontal gel electrophoresis apparatus (Sunrise™ 96), with a voltage at 5 V cm^{-1} of gel length (Thermo EC 3000-90 power supply, Thermo Electron Corporation, Milford, MA, United States) and a continuous gradient (TBE buffer 0.5×) for 3 hrs. The gels were visualized by UV light at 300 nm (Electronic UV Transilluminator mod. EB-40, Ultra Lum. Inc., Paramount, CA). Images were captured with a Photodocumentation System (Mod. DP001. FDC version 10; Vilber Lourmat, Marne la Vallée, France) and analyzed with a PhotoCapt MW (version 10.01 for Windows; Vilber Loumat, Marne la Vallée, France). *S. aureus* ATCC® 43300 (methicillin resistant *S. aureus*–MRSA), ATCC® 25923 (methicillin sensitive *S. aureus*–MSSA) and a maker of DNA (100 bp DNA, Ladder Cat. # 15628-019, Invitrogen) were used as controls.

Minimum Inhibitory Concentration (MIC) and determination of interactions between plant product and OXA

The MIC values were determined using the broth checkerboard microdilution assay on 96-well microplates (flat-bottom, Corning Inc., NY) containing 100 mL of MHB, following the CLSI document (M7-A6) [35, 36]. CLE or fractions (EtOAc or HX) were diluted into the wells (at final concentrations ranging from 4000 to 1.95 μg ml^{-1} [CLE], and from 500 to 0.244 μg ml^{-1} [fractions]), either alone or coupled with OXA (from 8000 to 3.9 μg ml^{-1}). *S. aureus* suspensions (cultures of ORSA [isolates] or OSSA [ATCC® 25923™] during the early exponential growth phase, after an overnight incubation on BHI) were centrifuged (900 × g for 6 min), the pellet diluted in sodium chloride 0.9%, and then spectrophotometrically adjusted at OD_{625} = 0.1 = 1 × 10^8 CFU mL^{-1} (further diluted, 1 × 10^5 CFU mL^{-1} final concentration). Afterwards, 10 μL of inoculums were added to the wells. The plates were then incubated at 37 °C for 24 hours. Finally, readings were performed visually as previously determined [7], wherein the presence of turbidity in the wells was considered indicative of microbial growth and a specific dye (resazurin 0.01%) was used to evaluate the metabolic activity of the microorganism. The MIC of the CLE, fractions (EtOAc or HX) or OXA alone, and all isoeffective combinations (association between plant product and OXA) were defined when the growth of the microorganism was inhibited at the lowest concentration. The growth control was composed of 100 μL of MHB and 10 μL of inoculum. The extract control was composed of 100 μL of MHB and 100 μL of the CLE or fraction (EtOAc or HX) and the sterility control contained only 100 μL of MHB.

Minimum Bactericidal Concentration (MBC)

The MBC was defined as the lowest concentration of OXA/extract/fractions that killed 99.9% of the original inoculums from each well where growth inhibition occurred in the analysis of MIC/Synergism. For each strain of ORSA and OSSA, MBC values were determined by removing 100 μl of microbial suspension from each well demonstrating no microbial growth and inoculating them with three serial dilutions of 1:10 on Petri plates (90 × 15 mm) containing the nutrient agar. Then, the plates were

incubated at 35 °C for 24 h. After that, colony counts were performed to determine which concentrations showed bactericidal or bacteriostatic effect.

Toxicity test: in vivo acute toxicity

Adult male Swiss mice weighing 30 to 45 g were obtained from the Central Animal Facility of the Federal University of Alfenas and housed in a controlled 12 h light/dark cycle at 23 °C and received water and food *ad libitum*. The acute toxicity in mice ($n = 10$) was performed with an oral dose of 5 g kg^{-1} of the CLE. During a seven-day period with adequate acclimatization (21 °C) and food and water given to the mice, *ad libitum*, the following parameters were assessed: hyperactivity, sedation, changes in stool characteristics (consistency), and food and water intake [37].

Data analysis

MIC and MBC values were from two replicate wells from two independent experiments. To assess the interactions between the CLE, HX, or EtOAc and OXA, the data obtained from the checkerboard microdilution assays were analyzed as previously described. The model-fractional inhibitory concentration index (FICI) was calculated as follows: FICA = MICAB/MICA and FICB = MICBA/MICB, where MICA and MICB are the MICs of samples A and B when acting alone, and MICAB and MICBA are the MICs of samples A and B when acting in combination, respectively. A and B represent the treatments (exposures) under investigation (i.e., A: can be an extract or fraction and B: OXA); then, FICI = FICA + FICB. "Synergy" was defined as an FICI ≤ 0.5, while "antagonism" was defined as an FICI value > 4.0. An FICI between 0.5 and 1.25 was considered to have an "additive effect" and between 1.25 and 4 "no interaction" [38, 39]. According to the ratio of MBC/MIC, the type of antimicrobial action displayed by each treatment was considered. If the ratio of MBC/MIC = 1 or 2, the effect was considered bactericidal, but if the ratio of MMC/MIC = 4 or 16, the effect was defined as bacteriostatic [40]. The selectivity index (SI) was calculated as follows: $SI = CC_{90}/MIC_{99.9}$, being CC_{90} adopted from our previous study [7].

Results

MIC and MBC values for OXA and the CLE, EtOAc, and HX from *T. paniculatum*

The MIC and MBC values for OXA and CLE, EtOAc, and HX against OSSA and the two clinical samples are shown in table 3. Increased MIC values were demonstrated for OXA against the isolates (samples G11.39 and G11.19, both resistant to this antibiotic [MIC ≥ 4 μg ml^{-1}]), confirming the ORSA profiles. We found MIC values of 500 μg ml^{-1} for EtOAc and HX and 4000 μg ml^{-1} for CLE against both OSSA and ORSA. The CLE was as effective as OXA against one of the isolates (sample G11.39). Both EtOAc and HX were more effective (lower MICs) than OXA against the two ORSA isolates.

Table 3. Minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations for the crude leaf extract (CLE) and fractions from *Talinum paniculatum* and for Oxacillin against *Staphylococcus aureus* and the types of antibacterial effects.

Microorganisms	*S. aureus* (ATCC 25923)				*S. aureus* (sample G11.39)				*S. aureus* (sample G11.19)			
Extract/ fraction/ antibiotic	MIC ($\mu g ml^{-1}$)	MBC ($\mu g ml^{-1}$)	MBC/ MIC	Antibacterial effect	MIC ($\mu g ml^{-1}$)	MBC ($\mu g ml^{-1}$)	MBC/ MIC	Antibacterial effect	MIC ($\mu g ml^{-1}$)	MBC ($\mu g ml^{-1}$)	MBC/ MIC	Antibacterial effect
CLE	4000	N	N/A	N/A	4000	N	N/A	N/A	4000	N	N/A	N/A
EtOAc	500	500	1	Bactericidal	500	N	N/A	N/A	500	N	N/A	N/A
HX	500	500	1	Bactericidal	500	N	N/A	N/A	500	N	N/A	N/A
Oxacillin	3.91	7.813	2	Bactericidal	4000	N	N/A	N/A	62.5	1000	16	Bacteriostatic

Antimicrobial Action: Bactericidal

Bacteriostatic

For plants products: MIC up to 100 $\mu g ml^{-1}$ = promising inhibitory potential, MIC between 100 and 625 $\mu g ml^{-1}$ = moderate inhibitory activity [7]. Fractions from the CLE of *Talinum paniculatum*; EtOAc = Ethyl acetate fraction and HX= Hexane fraction; *S. aureus:* samples G11.39 and G11.19 are from clinical environment*; S. aureus*: 5 ′ 10^5 CFU mL^{-1}; Antimicrobial resistance (AMR) classification: OXA MIC ≤ 2 $\mu g ml^{-1}$ = sensitive and ≥ 4 $\mu g ml^{-1}$ = resistant [35]. Ratio MBC/MIC = 1 or 2 → Effect was considered as bactericidal; Ratio MBC/MIC = 4 or 16 → Effect was defined as bacteriostatic [40]. N: inhibition of microbial growth was not observed at maximum used concentration; N/A: not applicable due to absence of an MBC value.

Antistaphylococcal effects for the CLE and fractions

In table 3, it is shown that the fractions EtOAc and HX showed bactericidal effects against OSSA and ORSA. MBC values were not observed for the CLE and fractions at maximum concentrations used in this study, 4000 $\mu g\,ml^{-1}$ and 500 $\mu g\,ml^{-1}$, respectively, against ORSA strains.

Final MIC values for the associations between plant product (CLE, EtOAc, and HX) and OXA, and FICI values from these associations

The associations between plant products (CLE, EtOAc, or HX from *T. Paniculatum*) and OXA, were investigated. As demonstrated in table 3, the treatment of OSSA or ORSA with CLE, EtOAc, or HX, resulted in outstanding MIC values (low MIC values). On the other hand, when the association between plant product (extract/fraction) and OXA was performed (table 4), OXA MICs increased 2- to 4092-fold, whereas the MICs of each fraction decreased up to 1020-fold. Taken together, data from tables 3 and 4 were interpreted as FICI values (table 4), which demonstrated that there was no synergy for any of the associations. The most common effect for the associations was "no interaction" (8 associations).

***In vivo*/*in vitro* toxicity data for *T. paniculatum* and selectivity index (SI)**

Of clinical relevance, the CLE from *T. paniculatum* did not present an acute toxicity at a dose of 5 $g\,kg^{-1}$ (table 5). In table 5, the results of the SI are also presented, and the best ones were from CLE against both OSSA (3.45) and ORSA (3.45).

Discussion

In this study, we first demonstrated that the CLE from *T. paniculatum* and its fractions (EtOA and HX) have antibacterial activity against OSSA and ORSA, and a bactericidal effect for the fractions against OSSA. Currently, resistance to ß-lactam antibiotics in *S. aureus* is a global and serious health threat, even in the clinical environment such as the healthcare settings, places where the ORSA strains used in this study were isolated [4, 41, 42]. Thus, new antimicrobials are urgently needed.

Over the last few decades, the screening of plants seems to be a good option for possible sources of new compounds with original antistaphylococcal action, including studies that evaluate the association between plant product and antibiotic [7, 43-45]. *T. paniculatum* was here evaluated because of previous studies about the antimicrobial activity against medically relevant strains of bacteria and fungi; the popular use in folk medicine, and the culinary use [7]. The antimicrobial activity for members of the *Talinaceae* family or related families have also been described [46, 47].

Table 4. Results for the contribution of each compound or plant product in the final MIC value during the association between (1) plant product (Crude leaf extract [CLE], EtOAc, or HX) from *Talinum paniculatum* and (2) Oxacillin against *Staphylococcus aureus*, correspondent Fractional inhibitory concentration index (FICI), and interpretation of the FICIs from the associations with the resulting type of interaction.

Microorganisms	***S. aureus* (ATCC 25923)**				***S. aureus* (sample G11.39)**				***S. aureus* (sample G11.19)**			
	Concentration ($\mu g\,ml^{-1}$)*		FICI		Concentration ($\mu g\,ml^{-1}$)		FICI		Concentration ($\mu g\,ml^{-1}$)		FICI	
Association	(1)	(2)		Interpretation	(1)	(2)		Interpretation	(1)	(2)		Interpretation
(1) CLE + (2) Oxacillin	4000	16000	>4	Antagonism	4000	16000	>4	Antagonism	4000	16000	>4	Antagonism
(1) EtOAc + (2) Oxacillin	0.49	7.8	2	No interaction	500	8000	3	No interaction	7.8	125	2	No interaction
(1) HX + (2) Oxacillin	0.49	7.8	2	No interaction	500	8000	3	No interaction	7.8	125	2	No interaction
(1) EtOAc + (2) HX	250	250	1	Additive effect	500	500	2	No interaction	500	500	2	No interaction

Interaction index interpretation: Antagonism; No interaction; Additive effect

Fractions from the CLE of *Talinum paniculatum*; EtOAc = Ethyl acetate fraction and HX = Hexane fraction; *Concentration of each substance in the associations between the (1) crude leaf extract (CLE)/fraction (EtOAc, or HX) from *Talinum paniculatum* and (2) oxacillin or (1) fraction and (2) fraction in which no microbial growth was observed; FICI data interpretation from the interactions/associations: 'synergy' (FICI ≤ 0.5), "Additive effect" (FICI > 0.5–1.25) 'no interaction' (FICI > 1.25–4.0), and 'antagonism' (FICI > 4.0) [38-39].

Table 5. Selectivity index (SI) for the crude leaf extract (CLE) and fractions, and *in vivo* toxicity data for the CLE from *Talinum paniculatum*.

Extract/ Fraction	**CLE**		**EtOAc**		**HX**	
Selectivity index						
	MIC	SI	MIC	SI	MIC	SI
S. aureus (ATCC 25923)	400	3.45	500	0.6	500	1.1
S. aureus (sample G11.39)	400	3.45	500	0.6	500	1.1
S. aureus (sample G11.19)	400	3.45	500	0.6	500	1.1
Toxicity	CLE		EtOAc		HX	
In vivo (mice)	Absence at 5 gkg^{1}		----		----	

SI: = $CC_{90}/MIC_{99.9}$; MIC: $\mu g\,ml^{-1}$; fractions from the CLE of *Talinum paniculatum*; EtOAc = ethyl acetate fraction and HX = hexane fraction.

As for the selectivity toward microbial cells, data have demonstrated a low toxicity for *T. paniculatum* leaves on other cells *in vitro*, justifying the antimicrobial potential of this plant [7]. We showed the selectivity of plant products from *T. paniculatum* toward *S. aureus* cells. Moreover, we found absence of an *in vivo* toxicity for *T. paniculatum* leaves at a dose of 5 $mg\,kg^{-1}$.

The screening for the antimicrobial activities of plant products alone, or in association with antibiotic, have been reported in myriad papers that document the effects against different microorganisms, including human pathogens [3, 30, 39, 40, 45, 48-51]. As seen in this study, decreased OXA lethality was observed for the association between plant products and antibiotic, and our results are in line with some previous reports [20, 31, 52]. About this context, the "one drug, one target, one disease" approach has for some time remained the conventional pharmaceutical approach to the search of drugs and treatment strategies. However, this paradigm has been gradually shifted toward the adoption of combination therapies as well as possible interactions between drug and food.

Contrarily to a desirable synergistic action between plant product and OXA, in this study was demonstrated that such associations caused an augmentation in OXA MICs. In this context, a putative action of antioxidants-containing plant product upon ROS levels should be considered. The action of some antibiotics have shown dependence on intense ROS accumulation and associated oxidative stress (i.e., OXA produces ROS

that promotes growth inhibition or kills *S. aureus*), and other studies have pointed that antioxidants-containing plant products and isolated antioxidant compounds can decrease the susceptibility of microorganisms to antimicrobials, including *S. aureus* to ß-lactam and other antistaphylococcal drugs [20, 31, 52-55].

Other evidences indicate a role of harnessing oxidative stress as an interesting way to enhance the killing efficacy of OXA and other antistaphylococcal drugs, regardless of macromolecular or drug-target interaction (the main antibiotic mode-of-action in interfering with cell wall biosynthesis), aside from the importance of new insights into the mechanisms behind signaling pathways in *S. aureus* to control the redox balance and develop high-level AMR/MDR [23, 56, 57]. A metabolic response of *S. aureus* toward oxidative stress caused by penicillin has been proposed through up-regulation of an environmental sensing and other gene, countering oxidative damage coupled with other effects generated by this ß-lactam antibiotic [29, 58]. Moreover, a successful response of *S. aureus vis-à-vis* oxidative stress imposed by antibiotics includes overproduction of antioxidant pigments, overexpression of detoxifying enzymes (such as superoxide dismutase [Sod], catalase and peroxiredoxin AhpC) and genes and enzymes related to DNA protection and repair (including *mrgA* response and pyrophosphohydrolase activity of MutT to remove oxidized guanine [8-oxoG or GO lesion] from the nucleotide pool), and an augmentation in protein damage repair (overexpression of thioredoxin).

It is well-known that ß-lactam antibiotics induce an increase in oxygen consumption to form superoxide ($O_2^{\bullet-}$) as a by-product via different pathways (such as the metabolism-related NADH depletion, the tricarboxylic acid (TCA) cycle, the electron transport chain, damage of iron sulphur clusters in proteins, and stimulation of the Fenton reaction). The production of $O_2^{\bullet-}$ allows the formation of other ROS, including hydrogen peroxide (H_2O_2), and the derivatives $HO^{\bullet}$ (via Fenton chemistry) and/or peroxinitrite ($ONOO^-$, via nitric oxide [•NO] biosynthesis pathways [58-60]. Moreover, while *S. aureus* Sod and catalases can enzymatically degrade $O_2^{\bullet-}$ and H_2O_2, respectively, no cellular detoxification mechanism is described for $HO^{\bullet}$, and this oxidant can avidly attack the DNA [59-61]. Thus, high-levels $HO^{\bullet}$ and the effects of blocking $HO^{\bullet}$ accumulation (controls) can interfere with MIC and MBC values of antistaphylococcus agents (including OXA), as previously reported [20]. In this context, these factors may, at least partially, explain the link between $HO^{\bullet}$ accumulation and oxidative DNA damage, as seen to a broader set of antibiotics and against different microorganisms [59-65], also explaining how antibiotic lethality is enhanced by amplifying basal ROS production and how antioxidants containing-plant products can interfere with this process [53]. In that way, we have reasons to believe that the prevention of OXA-induced ROS-mediated growth inhibition or killing of

S. aureus is related to a mechanism in which the levels/concentrations of compounds in *T. paniculatum* generate an effect "purely antioxidant" within *S. aureus* cells.

Taken together, our findings suggest that the alone CLE, HX and EtOAc from *T. paniculatum* present antistaphylococcal potential for furthers studies, *in vitro* involving other strains of *S. aureus* and *in vivo*. However, a word of warning should be issued regarding the *in vivo* association between *T. paniculatum* and OXA and the use of *T. paniculatum* should be phased out during OXA treatment. These *in vitro* effects carry *in vivo* implications and caution is required, since patients under OXA treatment parallel to the intake of leaves or herbal drug preparations from *T. paniculatum* may cause a decrease in OXA efficacy (jeopardizing therapeutic). Our data supports the fact that antioxidants containing-plant products, being a part of many nutrition products and medicines, may interact with OXA during antibiotic therapy and modify its action, causing resistance phenomenon or the failure of therapeutic regimens. However, to support this idea, these data should be investigated further, and the physician should take all this into account.

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Author contributions

Conceived and designed the experiments: CDC. Performed the experiments: CDC MFRN JJS LFCR. Analyzed the data: CDC. Contributed reagents/materials/analysis tools: MFGB GBS MRPLB. Gave technical support and conceptual advice: MFGB MRPLB. Wrote the paper: CDC. Supervised the study: CDC MRPLB. Final approval of manuscript: CDC JJS MFRN MFGB GBS LFCR MRPLB.

Disclosure statement

No potential conflict of interest was reported by the authors.

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