Research Article

In Vitro Synergistic Effect of Psidium guineense (Swartz) in Combination with Antimicrobial Agents against Methicillin-Resistant Staphylococcus aureus Strains

Tiago Gomes Fernandes,1 Amanda Rafaela Carneiro de Mesquita,1 Karina Perrelli Randau,2 Adelisa Alves Franchitti,3 and Eulâlia Azevedo Ximenes1

1 Laboratório de Fisiologia e Bioquímica de Microorganismos, Departamento de Antibióticos, Centro de Ciências Biológicas, Universidade Federal de Pernambuco, 50670-901 Recife, PE, Brazil
2 Laboratório de Farmacognosia, Departamento de Farmácia Universidade Federal de Pernambuco, Centro de Ciências da Saúde, 50670-901 Recife, PE, Brazil
3 Department of Biochemistry, Kansas State University, 141 Chalmers Hall, Manhattan, KS 66506, USA

Correspondence should be addressed to Eulâlia Azevedo Ximenes, eulaliaximenes@yahoo.com.br

Received 31 October 2011; Accepted 26 December 2011

Academic Editors: W. A. Gebreyes and T. Hatano

Copyright © 2012 Tiago Gomes Fernandes et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The aim of this study was to evaluate the antimicrobial activity of aqueous extract of Psidium guineense Swartz (Araçá-do-campo) and five antimicrobials (ampicillin, amoxicillin/clavulanic acid, cefoxitin, ciprofloxacin, and meropenem) against twelve strains of Staphylococcus aureus with a resistant phenotype previously determined by the disk diffusion method. Four S. aureus strains showed resistance to all antimicrobial agents tested and were selected for the study of the interaction between aqueous extract of P. guineense and antimicrobial agents, by the checkerboard method. The criteria used to evaluate the synergistic activity were defined by the fractional inhibitory concentration index (FICI). All S. aureus strains were susceptible to P. guineense as determined by the microdilution method. The combination of the P. guineense extract with the antimicrobial agents resulted in an eight-fold reduction in the MIC of these agents, which showed a FICI ranging from 0.125 to 0.5, suggesting a synergistic interaction against methicillin-resistant Staphylococcus aureus (MRSA) strains. The combination of the aqueous extract of P. guineense with cefoxitin showed the lowest FICI values. This study demonstrated that the aqueous extract of P. guineense combined with beta lactamics antimicrobials, fluoroquinolones, and carbapenems, acts synergistically by inhibiting MRSA strains.

1. Introduction

The widespread emergence of bacteria resistance to a large number of antimicrobial agents poses major health problems because of difficulties in treatment [1]. Staphylococcus aureus is an important pathogen both in community acquired and nosocomial infections and a common etiological agent of infections of many different tissues and organs (e.g., furuncle, carbuncle, abscess, myocarditis, endocarditis, pneumonia, meningitis, bacterial arthritis, and osteomyelitis) [2]. The multidrug resistant Staphylococcus aureus strains now pose serious problems to hospitalized patients and their care providers. The organism has successfully evolved numerous strategies for resisting, the action to practically all antimicrobial agents [3]. Thus, it is extremely important to find new antimicrobial agents or new ways for the treatment of infectious diseases caused by multidrug resistant microorganisms [4].

The screening of plant extracts and phytochemicals for antimicrobial activity has shown that higher plants, especially their secondary metabolites, are a source to provide structurally diverse bioactive compounds with different pharmacological activities, including antimicrobials [5].

According to Hemaiswarya et al. [6], plants-derived antimicrobials are less potent, which enhances the need to adopt a synergistic interaction between its bioactive compounds
to combat infections. Based on this knowledge, several studies have been published using crude plant extracts or phytochemicals combined with antimicrobial agents. These combinations can enhance the efficacy of the antimicrobial agents and are an alternative to treat infections caused by multidrug resistant microorganisms, which do not have any effective therapy available [4, 7, 8].

Psidium guineense Swartz (Myrtaceae), popularly known as wild guava, is a shrub native and widely dispersed throughout Tropical America. In Brazil, it mainly occurs across the coastline [9, 10]. Few studies have been focused on the phytochemical profile and pharmacological activity of this plant; however, it is widely used in folk medicine in regions of South America to treat infections of the gastrointestinal and genitourinary tract [11]. These authors associated the presence of mainly flavonoids and tannins in the fruits and leaves of P. guineense to be effective against Streptococcus mutans strains.

The aim of the present study was to evaluate the antimicrobial activity of the aqueous extract of P. guineense and to determine the synergistic potential of the combination of this extract with five antimicrobial agents against MRSA strains.

2. Materials and Methods

2.1. Plant Material and Extract Preparation. The plant materials used in this study consisted of Psidium guineense Swartz (leaves) that were collected in November/2009 from Moreno city, Pernambuco, Brazil, (08°07′07″S-35°05′32″W). This plant was identified by the biologist Olivia Cano, and the voucher specimen was deposited in the Herbarium Dârðano de Andrade Lima, Instituto Agronomico de Pernambuco and indexed under registration 83.564.

The leaves were air-dried for two weeks and then ground into fine powder using an electric dry mill. A total of 50 g of the powder was soaked in 1000 mL of boiled distilled water and extracted exhaustively at room temperature for 48 hours. The extracts were filtered through Whatman No. 2 filter paper. The aqueous extract was lyophilized to obtain a dry powder extract.

2.2. Phytochemical Screening. The preliminary phytochemical screening was carried out by various secondary metabolites present on aqueous extract of P. guineense using standard procedures [12–14]. The chromatographic analysis was made by TLC on Si gel (MERCK, Germany, 105553) developed by different solvent systems: (EtOAc–HCOOH–AcOH–H2O (100:11:11:26, v/v)) to investigate the presence of saponins, flavonoids, tannins, phenylpropanoids, alkaloids, condensed proanthocyanidins, leucoanthocyanidins, and iridoids and (n-BuOH–Me2CO-buffer phosphate pH = 5.0 (40:50:10 v/v)) to evaluate the presence of sugars.

2.3. Total Polyphenol Content (TPC). The total polyphenol content (TPC) was determined colorimetrically by using Folin–Ciocalteu’s reagent and expressed as pyrogallol, according to the Brazilian Pharmacopoeia with modifications [15]. Thus, samples of aqueous extract of P. guineense (1000 µg/mL) were prepared in distilled water. Concentrations (10 to 30 µg/mL) were measured. The standard pyrogallol (1000 µg/mL) was prepared in distilled water. 80 microliters of each solution was transferred to a 25 mL flask containing distilled water (10 mL) and Folin–Ciocalteu’s reagent (1 mL). The volume was completed with anhydrous sodium carbonate solution (10.75% w/v), resulting in final concentrations of 3.2 µg/mL of pyrogallol. A standard curve was determined with concentrations between 1.6 and 4.8 µg/mL ($r^2 = 0.999$). Final results were given as pyrogallol equivalents. The samples were scanned in a UV/VIS spectrophotometer (Evolution 60S, Thermo Scientific), and measurements were obtained 15 min after addition of the sodium-carbonate solution and scanning ranging from 500 to 900 nm wavelength. Distilled water was used as a blank. All measurements were performed in triplicate.

2.4. Bacterial Strains and Inoculum Standardization. S. aureus strains ($n = 11$) were isolated from clinical specimens and food. The standard strain used was S. aureus ATCC 25923. Strains were isolated in sheep blood agar and after identification they were stored in brain heart infusion (BHI) plus glycerol 20% v/v [16]. The S. aureus strains used in this study showed a resistant phenotype, by the diffusion method, to several antimicrobial agents such as beta-lactams, aminoglycosides, macrolides, fluoroquinolones, tetracycline, chloramphenicol, and lincosamides. These strains were cultured onto Mueller Hinton Agar (MHA) (Acumedia Manufacturers, Baltimore, USA) and incubated at 37°C for 18 hours. Single colonies were selected and inoculated into Mueller–Hinton broth (Acumedia Manufacturers, Baltimore, USA) to turbidity comparable to that of 0.5 McFarland standard, which is equivalent to a bacterial count of approximately $10^8$ CFU/mL.

After that, the bacterial suspension was diluted in saline (1:10) to obtain a final inoculum of $10^7$ CFU/mL.

2.5. Antimicrobial Agents. The standard reference powders of ampicillin; amoxicillin/clavulanic acid; cefoxitin; ciprofloxacin; gentamicin; meropenem were provided by Eurofarma Laboratório LTDA, Brazil. Resistance was defined for each case: ampicillin (AMP, MIC $\geq 0.25$ µg/mL); amoxicillin/clavulanic acid (AMC, MIC $\geq 8$ µg/mL); cefoxitin (CFO, MIC $\geq 4$ µg/mL); ciprofloxacin (CIP, MIC $\geq 4$ µg/mL); gentamicin (GEN, MIC $\geq 8$ µg/mL; meropenem (MER, MIC $\geq 16$ µg/mL).

2.6. Antimicrobial Activity. The Minimal Inhibitory Concentration (MIC) test was performed by the microdilution broth method, following the recommendations established by Clinical Laboratory Standards Institute, [17], with some modifications. Serial two-fold dilutions of aqueous extract of P. guineense and antimicrobial agents were prepared in sterile 96-well microplates containing Mueller Hinton broth (MHB). Five microliters of bacterial suspension were inoculated in each well to give a final concentration of $10^6$ CFU/mL. P. guineense extract and antimicrobial agents concentrations ranged from 7.25 to 1000 µg/mL and 3.12 to
400 µg/mL, respectively. The growth inhibition was demonstrated by optical density at 630 nm using a microplate reader (Thermo plate—TP Reader). Considering the total growth (100%) in the control well (MHB + bacteria), the percentage of growth reduction was attributed to the remaining wells. The MIC was reported as the lowest concentration of *P. guineense* extract or antimicrobial agents that inhibited the bacterial growth after 24 h of incubation at 37°C. In order to determine the Minimal Bactericidal Concentration (MBC), the contents of the well that showed higher or equal than 70% of growth inhibition were seeded into MHA. After 24 h of incubation at 37°C, the number of surviving *S. aureus* was determined. The MBC was defined as the lowest extract concentration at which 99.9% of the bacteria have been killed. All experiments were carried out in duplicate on two different days.

2.7. Determination of In Vitro Synergistic Activity. Combinations of *P. guineense* and antimicrobial agents were tested by the checkerboard method. The appropriate dilution of *P. guineense* extract and antimicrobial agents were performed into MHB. From these dilutions, one hundred microliters were added in 96-well microplates to obtain a final concentration equal to MIC or six dilutions lower than MIC to *P. guineense* and nine dilutions lower than MIC to antimicrobial agents. Each well received 5 µL of the bacterial suspensions (10⁵ CFU/mL). Plates were incubated for 24 hours. Interpretation of the data was achieved by calculating the fractional inhibitory concentration index (FICI) as follows: (MIC of *P. guineense* in combination with antimicrobial agents/MIC of *P. guineense*) + (MIC of antimicrobial agents in combination with *P. guineense*/MIC of antimicrobial agents). The combination was considered to be synergistic when the FICI was ≤0.5, additive when it was 0.5 to ≤1, and antagonistic when ≥2 [18].

3. Results

3.1. Phytochemical Profile. The phytochemical profile from the *Psidium guineense* extract showed the presence of tannins, flavonoids, condensed proanthocyanidins, leucoanthocyanidins, and sugar. On the other hand, the presence of alkaloids, phenylpropanoid, and saponins was not verified. The aqueous extract of *P. guineense* yielded 3.7% (w/w) over 50 g of plant material.

Concerning the total polyphenol content, the results of this study suggest that the aqueous extract of *P. guineense* show an important amount of such secondary metabolites. Thus, the total polyphenol content was 21.62 ± 0.40 g% (1.51%) as pyrogallol equivalent.

3.2. Antimicrobial Activity. MIC and MBC values of *P. guineense* extract and of the ampicillin, amoxicillin/clavulanic acid, cefoxitin, ciprofloxacin, gentamicin, and meropenem against twelve *S. aureus* strains are shown in Table 1.

The aqueous extract of *P. guineense* showed a strong activity against all *S. aureus* strains with MIC values between 250 and 500 µg/mL. The *S. aureus* strains revealed a resistance profile against most antimicrobial agents tested, in particular to the beta-lactam antibiotics. The MIC values for ampicillin and cefoxitin ranged from 3.12 to 400 µg/mL which showed to be less effective against the *S. aureus* strains tested. For amoxicillin/clavulanic acid, the values ranged from 3.12 to 50 µg/mL. Among all *S. aureus* strains tested, seven showed to be resistant to ciprofloxacin. For meropenem, MIC values ranged from 3.12 to 25 µg/mL. All strains showed to be sensitive to gentamicin, except LFBM 26, LFBM 28, LFBM 33. Four strains of *S. aureus* (LFBM 01, LFBM 26, LFBM 28, LFBM 33) showed resistance to all antimicrobial agents tested. This resistance profile selected the microorganisms for the study of the interaction between *P. guineense* extract and antimicrobial agents. The values of MBC were higher than MIC in one dilution.

3.3. Determination of In Vitro Synergistic Activity. The MICs obtained by the combination of the aqueous extract of *P. guineense* with ampicillin, amoxicillin/clavulanic acid, cefoxitin, ciprofloxacin, and meropenem, against *S. aureus* strains (LFBM 01, LFBM 26, LFBM 28, LFBM 33) are listed in Table 2. The minimal inhibitory concentrations of *P. guineense* extract (250 µg/mL) enhanced the antistaphylococcal activity of all antimicrobial agents. The synergistic activity was detected by an eight-fold decrease in the MIC of the antimicrobial agents in the combination and determined by the FICI ≤0.5.

The combination of the aqueous extract of *P. guineense* and cefoxitin showed the lowest FICI whose values ranged from 0.125 to 0.5. The MIC of cefoxitin (individual MIC 100–400 µg/mL) was lowered to 1/512 (combined MIC ranged 0.19–0.78 µg/mL), when it was used in combination with the aqueous extract of *P. guineense* (MIC 250 µg/mL). For *S. aureus* LFBM 26 and LFBM 33 strains, this combination was also efficient on reducing the MIC of *P. guineense* extract to 1/8 × MIC (31.25 µg/mL) or 1/4 × MIC (62.25 µg/mL), respectively.

The inhibition of the growth of all the *S. aureus* strains by meropenem was enhanced by *P. guineense* extract (FICI 0.5). This combination was more effective against LFBM 28 (FICI 0.25) which individual MIC of *P. guineense* (250 µg/mL) lowered to 1/4 × MIC.

For *S. aureus* LFBM 33 strain, the MIC value of ciprofloxacin (individual MIC 50 µg/mL) was lowered to 0.78 µg/mL when combined with *P. guineense* extract (1/4 × MIC) enhancing the antistaphylococcal activity of this fluoroquinolone (FICI 0.25). All other combinations of the aqueous extract of *P. guineense* with antimicrobial agents demonstrated synergistic activity, enhancing its activity, with FICI 0.5.

4. Discussion

Phytochemicals have great potential as antimicrobial compounds and have been proven to have great therapeutic potential [19]. Some secondary metabolites have the ability to increase the susceptibility of the microorganism. When used in combination, these metabolites have the potential to either inhibit the modified targets or exhibit a synergy by
### Table 1: MIC/MBC of the aqueous extract of *Psidium guineense* and antimicrobial agents against MRSA strains.

| Staphylococcus aureus | MIC/MBC (µg/mL) | Resistance phenotype |
|-----------------------|-----------------|----------------------|
|                        |                  | Control strain       |
| ATCC 25923            | 3.12/6.24       |                      |
| LFBM 01               | 25.0/50.0       | AMC, CFO, CIP, GET   |
| LFBM 05               | 6.25/12.5       | 12.5/25.0/250/500    |
| LFBM 08               | 3.12/6.24       | 12.5/25.0/250/500    |
| LFBM 16               | 12.5/25.0       | 12.5/25.0/250/500    |
| LFBM 26               | 50.0/100        | 25.0/50.0/250/500    |
| LFBM 28               | 25.0/50.0       | 25.0/100/250/500     |
| LFBM 29               | 12.5/25.0       | 12.5/25.0/250/500    |
| LFBM 30               | 12.5/25.0       | 12.5/25.0/250/500    |
| LFBM 31               | 12.5/25.0       | 12.5/25.0/250/500    |
| LFBM 32               | 12.5/25.0       | 12.5/25.0/250/500    |
| LFBM 33               | 12.5/25.0       | 12.5/25.0/250/500    |

| LFBM 01               | 25.0/50.0       | 25.0/100/250/500     |
| LFBM 05               | 6.25/12.5       | 12.5/25.0/250/500    |
| LFBM 08               | 3.12/6.24       | 12.5/25.0/250/500    |
| LFBM 16               | 12.5/25.0       | 12.5/25.0/250/500    |
| LFBM 26               | 50.0/100        | 25.0/50.0/250/500    |
| LFBM 28               | 25.0/50.0       | 25.0/100/250/500     |
| LFBM 29               | 12.5/25.0       | 12.5/25.0/250/500    |
| LFBM 30               | 12.5/25.0       | 12.5/25.0/250/500    |
| LFBM 31               | 12.5/25.0       | 12.5/25.0/250/500    |
| LFBM 32               | 12.5/25.0       | 12.5/25.0/250/500    |
| LFBM 33               | 12.5/25.0       | 12.5/25.0/250/500    |

MIC: minimal inhibitory concentration; MBC: minimal bactericidal concentration; ATCC: American Type Culture Collection; LFBM: Laboratório de Fisiologia e Bioquímica de Microorganismos; AMC: amoxicillin/clavulanic acid; AMP: ampicillin; AZI: azithromycin; CFO: cefoxitin; CIP: ciprofloxacin; GET: gentamicin; PEN: penicillin; MER: meropenem; AE: aqueous extract of *P. guineense*.

1Resistence phenotype determined by the disk-di

### Table 2: Combination testing of the aqueous extract of *Psidium guineense* with antimicrobial agents against MRSA strains.

| Staphylococcus aureus | Combination | Individual MIC (µg/mL) | Combination MIC (µg/mL) | Individual | FIC index (FICI) | Interpretation | % MIC reduced | % reduction of viable cells¹ |
|-----------------------|-------------|------------------------|-------------------------|------------|-----------------|----------------|---------------|-----------------------------|
| LFBM 01               | AE + AMC    | 125/0.048              | 0.5/0.002               | Synergistic | 50.0/99.8       | 80.21          |
| LFBM 05               | AE + AMP    | 125/0.78               | 0.5/0.002               | Synergistic | 50.0/99.8       | 84.43          |
| LFBM 08               | AE + CFO    | 125/0.78               | 0.5/0.002               | Synergistic | 50.0/99.8       | 87.04          |
| LFBM 26               | AE + CIP    | 125/0.09               | 0.5/0.002               | Synergistic | 50.0/99.8       | 93.49          |
| LFBM 28               | AE + MER    | 125/0.048              | 0.5/0.002               | Synergistic | 50.0/99.8       | 84.24          |
| LFBM 30               | AE + AMC    | 125/0.78               | 0.5/0.002               | Synergistic | 50.0/99.8       | 88.82          |
| LFBM 31               | AE + AMP    | 125/0.78               | 0.5/0.002               | Synergistic | 50.0/99.8       | 83.58          |
| LFBM 32               | AE + CFO    | 125/0.78               | 0.5/0.002               | Synergistic | 50.0/99.8       | 87.04          |
| LFBM 33               | AE + CIP    | 125/0.09               | 0.5/0.002               | Synergistic | 50.0/99.8       | 94.59          |
| LFBM 01               | AE + MER    | 125/0.048              | 0.5/0.002               | Synergistic | 50.0/99.8       | 84.24          |

MIC: minimal inhibitory concentration; FIC: fractional inhibitory concentration; AMC: amoxicillin/clavulanic acid; AMP: ampicillin; CFO: cefoxitin; CIP: ciprofloxacin; GET: gentamicin; PEN: penicillin; MER: meropenem; AE: aqueous extract of *P. guineense*; LFBM: Laboratório de Fisiologia e Bioquímica de Microorganismos.

¹% reduction of viable cells in wells containing the combination aqueous extract of *P. guineense* (1/2 MIC) and antimicrobial agents (1/512 MIC).
blocking one or more of the targets in the metabolic pathway, acting as a modifier of multidrug resistance mechanisms [6].

Several studies have investigated the interactions between antibiotics and phytochemicals or crude extracts against MRSA strains [4, 7, 8, 20].

In the Myrtaceae family, there is a large variety of phytochemicals with antimicrobial activity which include terpenes present in essential oils, flavonoids [21], and tannins [22].

The genus Psidium is native to America but is distributed worldwide. Their specimens are traditionally used in the prevention or treatment of a large number of diseases. Preparations of twigs or leaves of Psidium species are extensively used for the control of gastrointestinal and respiratory disorders as well as in the treatment of skin damage [23].

Ethnopharmacological evaluation demonstrated the antimicrobial activities of Psidium ssp. that explain its use for the treatment of infectious diseases of the digestive, respiratory, urinary tract as well as the skin and soft tissues. Within this genus, P. guajava is the most studied and used species [24]. Thus, we are interested in P. guineense, an indigenous plant of Brazil, because it is characterized by its ample spectrum of uses including gastrointestinal disorders, infection of the genitourinary tract, treatment of colds, bronchitis, and ulcers [25].

According to Sartoratto et al. [26], strong activity is for MIC values between 50–500 µg/mL, moderate activity MIC values between 600–1500 µg/mL, and weak activity above 1500 µg/mL. Comparing with literature results, the aqueous extract of P. guineense (250–500 µg/mL) has a strong activity against MRSA strains tested.

The phytochemical analysis from the leaves of P. guineense indicated the presence of flavonoids, hydrolysable, and condensed tannins distributed throughout the leaf tissue [27]. These results are in agreement with those obtained in the present study. Total polyphenol content 21.62 ± 0.40 g% determined in aqueous extract of Psidium guineense plays an important role on biological properties of this extract.

Neira González et al. [11] associated the presence of flavonoids, mainly avicularin, quercetin, and guaijaverin present in the ethanolic extract of P. guineense, with the activity against clinical isolated Streptococcus mutans strains.

Flavonoids have the ability to complex with proteins and bacterial cells forming irreversible complexes mainly with nucleophilic amino acids. This complex often leads to inactivation of the protein and loss of its function [27].

Tannins are not crystallizable substances and when they are in the aqueous system, form colloidal solutions. The antimicrobial activity of tannins can be summarized as follows: (i) binding with proteins and adhesins, inhibiting enzymes, (ii) complexation with the cell wall and metal ions, and (iii) disruption of the plasmatic membrane [28].

Synergism of natural products and antimicrobial agents is a thrust area of phyto medicinal research, developing novel perspective of phytopharmaceuticals. The synergism of plant-derived compounds and antimicrobial agents has been evaluated previously against pathogenic microorganisms. The approach is not exclusive for extract combinations, since effective combinations between single natural products, essential oils or extracts with chemosynthetics or antibiotics have been described [6, 29].

In addition to achieving these synergistic effects, the combinations of two or more compounds are essential for the following reasons: (1) to prevent or suppress the emergence of resistant strains, (2) to decrease dose-related toxicity, as a result dosage, and (3) to attain a broad spectrum of activity [18].

In this study, a growth inhibitory effect of P. guineense extract on MRSA strains was observed for its combinations with beta-lactams, carbapenems, and fluoroquinolones.

The combination of P. guineense extract and beta-lactam may help to reduce the amount of antimicrobial agents used and deliver a medicine with similar or greater potency as antimicrobial. More importantly, since phytochemicals are structurally different from antimicrobial agents and often have different modes of action, they may provide new means of studying the mechanisms of bacterial control at a molecular level. With the increase prevalence of multidrug resistant S. aureus, synergism testing using various combinations of phytochemicals with antimicrobial agents could be a powerful tool in helping to select appropriate antimicrobial therapy [6, 29].

The indiscriminate use of antimicrobial agents in the treatment of bacterial infections has led to the emergence and spread of resistant strains, and it resulted in a great loss of clinical efficacy of previously effective first-line antimicrobials which results in shifting of antimicrobial treatment regimen to second-line or third-line antimicrobial agents that are more expensive with many side effects [30]. In fact, studies have showed that crude extracts of plants possess the ability to enhance the activity of antimicrobial agents [4, 7, 8].

There is a wide list of phytochemicals which act as inhibitors, and a few of them are glycosylated flavones suppressing topoisomerase IV activity, myricetin inhibiting DnA helicase, allicin inhibiting RNA synthesis. Corilagin, a polyphenol from Arctostaphylos uva-ursi is found to markedly reduce the MIC of beta-lactam agents against MRSA. According to Hemaiswarya et al. [6], there are two possibilities regarding the mechanism of action of corilagin, namely, inhibition of penicillin binding protein (PBP2a) activity or inhibiting its production.

The polyphenol epigallocatechin gallate (EGCg) from green tea is believed to be synergic with beta-lactam agents since both attacked the same target site, namely, peptidoglycan. EGCg inhibits the penicillinase produced by S. aureus thereby restoring the activity of penicillin. The combination of EGCg with ampicillin/sublactam reduced the MIC30 to 4 µg/mL from its initial value of 16 µg/mL [6].

Hatano et al. [31] investigated the synergistic activity of two proanthocyanidins isolated from the fruits of the Zizyphus genus. Although the MICs of these polyphones were 512–1024 µg/mol, both reduced the MIC for oxacillin to 1/2–1/16 of those in the absence of the polyphenols.

It is interesting to note that most plant secondary metabolites have weak antimicrobial activity, several orders of magnitudes less than that of common antimicrobial agents
produced by bacteria and fungi. In spite of the fact that plant-derived antimicrobials are less potent, plants fight infections successfully. Hence, it becomes apparent that plants adopts a synergistic mechanism between their compounds [29].

According to Rosales-Reyes et al. [32], the use of the crude extract in our study was intentional and based on the belief that it would be the closest representation to that of traditional preparations.

Therefore, it is speculated that the efficacy of a crude extract may be due to the interplay between the different active constituents that may be present in the extract leading to better activity and/or decrease in potential toxicity of some individual constituents [33].

5. Conclusion

An antibacterial effect of *P. guineense* extract and a synergistic effect in combination with antimicrobial agents were reproducibly observed and might be an interesting alternative therapy for infectious diseases caused by MRSA strains. In addition, more studies, including toxicity tested *in vivo*, need to be conducted on this plant before therapeutic treatments are implemented.

Acknowledgments

The authors thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting the fellowship as well as the financial support and infrastructure provided by the Universidade Federal de Pernambuco (UFPE) which made this study possible.

References

[1] M. Guzmán-Blanco, C. Mejía, R. Isturiz et al., “Epidemiology of meticillin-resistant *Staphylococcus aureus* (MRSA) in Latin America,” *International Journal of Antimicrobial Agents*, vol. 34, no. 4, pp. 304–308, 2009.

[2] A. Nostro, A. R. Blanco, M. A. Cannatelli et al., “Susceptibility of methicillin-resistant staphylococci to oregano essential oil, carvacrol and thymol,” *FEMS Microbiology Letters*, vol. 230, no. 2, pp. 191–195, 2004.

[3] M. E. Mulligan, K. A. Murray-Leisure, B. S. Ribner et al., “Methicillin-resistant *Staphylococcus aureus*: a consensus review of the microbiology, pathogenesis, and epidemiology with implications for prevention and management,” *American Journal of Medicine*, vol. 94, no. 3, pp. 313–328, 1993.

[4] G. M. Adwan, B. A. Abu-Shanab, and K. M. Adwan, “In vitro activity of certain drugs in combination with plant extracts against *Staphylococcus aureus* infections,” *Pakistan Journal of Medical Sciences*, vol. 24, no. 4, pp. 541–544, 2008.

[5] P. Y. Chung, P. Navaratnam, and L. Y. Chung, “Synergistic antimicrobial activity between pentacyclic triterpenoids and antibiotics against *Staphylococcus aureus* strains,” *Annals of Clinical Microbiology and Antimicrobials*, vol. 10, article 25, 2011.

[6] S. Hemaiswarya, A. K. Kruthiveni, and M. Doble, “Synergism between natural products and antibiotics against infectious diseases,” *Phytomedicine*, vol. 15, no. 8, pp. 639–652, 2008.

[7] J. E. C. Betoni, R. P. Mantovani, L. N. Barbosa, L. C. Di Stasi, and A. Fernandes, “Synergism between plant extract and antimicrobial drugs used on Staphylococcus aureus diseases,” *Memorias do Instituto Oswaldo Cruz*, vol. 101, no. 4, pp. 387–390, 2006.

[8] C. O. Esimone, I. R. Iroha, E. C. Ibezie, C. O. Okeh, and E. M. Okpana, “In vitro evaluation of the interaction between tea extracts and penicillin *G* against staphylococcus aureus,” *African Journal of Biotechnology*, vol. 5, no. 11, pp. 1082–1086, 2006.

[9] M. Pio Corrêa, *Dicionário das plantas úteis do Brasil e das exóticas cultivadas*, Ministério da Agricultura, Instituto Brasileiro de Desenvolvimento Florestal, Rio de Janeiro, Brazil, 2nd edition, 1984.

[10] J. A. Silva, D. B. Silva, N. T. V. Junqueira, and L. R. N. Andrade, *Frutas nativas dos cerrados*, EMBRAPA, Centro de Pesquisa Agropecuária dos Cerrados-CPAC, Brasília, Brazil, 1994.

[11] A. M. Neira González, M. B. Ramírez González, and N. L. Sánchez Pinto, “Phytochemical study and antibacterial activity of Psidium guineense *Sw* (choba) against *Streptococcus mutans*, causal agent of dental caries,” *Revista Cubana de Plantas Medicinales*, vol. 10, no. 3–4, 2005.

[12] E. A. H. Roberts, R. A. Cartwright, and D. J. Wood, “Flavonoids of tea,” *Journal of the Science of Food and Agriculture*, vol. 7, pp. 637–646, 1956.

[13] H. Wagner and S. Bladt, *Plant Drug Analysis*, Springer, New York, NY, USA, 2nd edition, 1996.

[14] J. B. Harborne, *Phytochemical Methods*, Chapman & Hall, London, UK, 3rd edition, 1998.

[15] December 2011, [http://www.anvisa.gov.br/farmacopeiabrasileira/index.htm](http://www.anvisa.gov.br/farmacopeiabrasileira/index.htm).

[16] E. W. Koneman et al., *Diagnóstico microbiológico: texto e atlas colorido*, Guanabara Koogan, Rio de Janeiro, Brazil, 6th edition, 2008.

[17] CLSI—Clinical Laboratory Standards Institute, “Performance Standards for Antimicrobial Susceptibility Testing M100-S20,” 2010.

[18] G. M. Eliopoulos and R. C. Moellering Jr., “Antimicrobial combinations,” in *Antibiotics in Laboratory Medicine*, pp. 330–338, Williams & Wilkins, Baltimore, Md, USA, 4th edition, 1996.

[19] M. Stavri, L. J. V. Piddock, and S. Gibbons, “Bacterial efflux pump inhibitors from natural sources,” *Journal of Antimicrobial Chemotherapy*, vol. 59, no. 6, pp. 1247–1260, 2007.

[20] S. Gibbons, “Anti-staphylococcal plant natural products,” *Natural Product Reports*, vol. 21, no. 2, pp. 263–277, 2004.

[21] N. E. Hernández, M. L. Tereschuk, and L. R. Abdala, “Antimicrobial activity of flavonoids in medicinal plants from Tafi del Valle (Tucuman, Argentina),” *Journal of Ethnopharmacology*, vol. 73, no. 1–2, pp. 317–322, 2000.

[22] C. D. Djipa, M. Delmée, and J. Quetin-Leclercq, “Antimicrobial activity of bark extracts of *Syzygium jambos* (L.) Alston (Myrtaceae),” *Journal of Ethnopharmacology*, vol. 71, no. 1–2, pp. 307–313, 2000.

[23] A. Caceres, L. Fletes, L. Aguilar et al., “Plants used in Guatemala for the treatment of gastrointestinal disorders. 3. Confirmation of activity against enterobacteria of 16 plants,” *Memorias do Instituto Oswaldo Cruz*, vol. 94, no. 3, pp. 313–328, 1993.

[24] L. C. Di Stasi and C. A. Hiruma-Lima, “An antibacterial effect of *P. guineense* extract and a synergistic effect in combination with antimicrobial agents were reproducibly observed and might be an interesting alternative therapy for infectious diseases caused by MRSA strains. In addition, more studies, including toxicity tested *in vivo*, need to be conducted on this plant before therapeutic treatments are implemented.

Acknowledgments

The authors thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting the fellowship as well as the financial support and infrastructure provided by the Universidade Federal de Pernambuco (UFPE) which made this study possible.

References

[1] M. Guzmán-Blanco, C. Mejía, R. Isturiz et al., “Epidemiology of meticillin-resistant *Staphylococcus aureus* (MRSA) in LatinAmerica,” *International Journal of Antimicrobial Agents*, vol. 34, no. 4, pp. 304–308, 2009.

[2] A. Nostro, A. R. Blanco, M. A. Cannatelli et al., “Susceptibility of methicillin-resistant staphylococci to oregano essential oil, carvacrol and thymol,” *FEMS Microbiology Letters*, vol. 230, no. 2, pp. 191–195, 2004.

[3] M. E. Mulligan, K. A. Murray-Leisure, B. S. Ribner et al., “Methicillin-resistant *Staphylococcus aureus*: a consensus review of the microbiology, pathogenesis, and epidemiology with implications for prevention and management,” *American Journal of Medicine*, vol. 94, no. 3, pp. 313–328, 1993.

[4] G. M. Adwan, B. A. Abu-Shanab, and K. M. Adwan, “In vitro activity of certain drugs in combination with plant extracts against *Staphylococcus aureus* infections,” *Pakistan Journal of Medical Sciences*, vol. 24, no. 4, pp. 541–544, 2008.

[5] P. Y. Chung, P. Navaratnam, and L. Y. Chung, “Synergistic antimicrobial activity between pentacyclic triterpenoids and antibiotics against *Staphylococcus aureus* strains,” *Annals of Clinical Microbiology and Antimicrobials*, vol. 10, article 25, 2011.

[6] S. Hemaiswarya, A. K. Kruthiveni, and M. Doble, “Synergism between natural products and antibiotics against infectious diseases,” *Phytomedicine*, vol. 15, no. 8, pp. 639–652, 2008.
[26] A. Sartoratto, A. L. M. Machado, C. Delarmelina, G. M. Figueira, M. C. T. Duarte, and V. L. G. Rehder, "Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil," Brazilian Journal of Microbiology, vol. 35, no. 4, pp. 275–280, 2004.

[27] R. B. Ferreira et al., “Morphoanatomy, histochemistry and phytochemistry of Psidium guineense Sw. (Myrtaceae) Leaves,” Journal of Pharmacy Research, vol. 4, no. 4, pp. 942–944, 2011.

[28] M. M. Cowan, “Plant products as antimicrobial agents,” Clinical Microbiology Reviews, vol. 12, no. 4, pp. 564–582, 1999.

[29] H. Wagner and G. Ulrich-Merzenich, “Synergy research: approaching a new generation of phytopharmaceuticals,” Phytomedicine, vol. 16, no. 2-3, pp. 97–110, 2009.

[30] S. Mandal, M. DebMandal, N. K. Pal, and K. Saha, “Synergistic anti-Staphylococcus aureus activity of amoxicillin in combination with Emblica officinalis and Nymphae odorata extracts,” Asian Pacific Journal of Tropical Medicine, vol. 3, no. 9, pp. 711–714, 2010.

[31] T. Hatano, M. Kusuda, K. Inada et al., “Effects of tannins and related polyphenols on methicillin-resistant Staphylococcus aureus,” Phytochemistry, vol. 66, no. 17, pp. 2047–2055, 2005.

[32] T. Rosales-Reyes, M. de la Garza, C. Arias-Castro et al., "Aqueous crude extract of Rhoeo discolor, a Mexican medicinal plant, decreases the formation of liver preneoplastic foci in rats," Journal of Ethnopharmacology, vol. 115, no. 3, pp. 381–386, 2007.

[33] T. Birdi, P. Daswani, S. Brijesh, P. Tetali, A. Natu, and N. Antia, “Newer insights into the mechanism of action of Psidium guajava L. leaves in infectious diarrhoea,” BMC Complementary and Alternative Medicine, vol. 10, article no. 33, 2010.