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Modulating antibacterial activity against multidrug-resistant *Escherichia coli* and *Staphylococcus aureus* of the flavonoid pectolinarin isolated from *Lantana camara* leaves

Abstract

The antibiotic potentiating activity against standard and multidrug-resistant *Escherichia coli* and *Staphylococcus aureus* bacterial strains of the natural compound 5"-O-α-L-raminopyranosyl-1'-β-D-glycopyranosyl-4',6-dimethoxyflavone (pectolinarin) isolated from *L. camara leaves* was evaluated. Tests for antibacterial activity of the pure natural substance and analysis of the potentiation of antibacterial activity of pectolinarin associated with antibiotics were carried out against standard and multiresistant bacterial strains of *Escherichia coli* and *Staphylococcus aureus* by microdilution. Pectolinarin, when combined with the antibiotic gentamicin, showed synergism, potentiating growth inhibition against Gram-positive *S. aureus* strains. The pectolinarin flavonoid when combined with the gentamicin antibiotic potentiated its action Gram-positive *S. aureus* bacteria. Moreover, an antagonistic effect was observed when the pectolinarin was combined with the penicillin antibiotic against the multiresistant *S. aureus* 358 strain. This research suggests that pectolinarin is a compound with potential application as an antibacterial drug.

Keywords: flavonoid, *Lantana camara*, antibacterial activity

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Introduction

Flavonoids are one of the most diverse and important classes among plant chemical constituents, with over 6,000 flavonoids described.[1–3] Flavonoids present as their most significant subclasses: anthocyanins, catechins, chalcones, dihydroflavonols, flavonols, flavones, flavanones, and isoflavones.[4] In plants, certain functions such as protection against pathogenic microorganisms, antioxidant action, allelopathic action, enzymatic inhibition, and protection against the incidence of ultraviolet rays are attributable to flavonoids.[5–8] From the flavonoid subclasses, flavones stand out given their protective action, protecting plant cells from damage caused by photooxidation,[9] as well as protecting the body against free radical species.[10] Substances extracted from plants have been arousing much scientific interest due to the diversity of the biological activities they present.[11–15] One such plant species that has been of interest to the scientific community is the *L. camara* from the Verbenaceae family, originating from the Americas and Africa.[16] *L. camara* is a plant that possesses a toxin, which considering its toxicity, has been used as a repellent against *Aedes* mosquito larvae.[17] Medicinal plants are an important source for obtaining new molecules. While several synthesis methods are used to get effective drugs to treat certain diseases, plants can produce chemical constituents that are difficult to obtain through synthesis procedures.[18] In this context, obtaining new substances isolated from medicinal plants and investigating their structural and pharmacological properties is essential. In the present study, the antibiotic potentiating activity against standard and multidrug-resistant *Escherichia coli* and *Staphylococcus aureus* bacterial strains of the natural compound 5"-O-α-L-raminopyranosyl-1'-β-D-glycopyranosyl-4',6-dimethoxyflavone (pectolinarin) (Figure 1) isolated from *L. camara leaves* was evaluated.

Materials and methods

Plant material

Leaves from *L. camara* were collected in Vale do Alemão site in Pernambuquinho, Guaramiranga-Ceará (Brazil) district (S4°12'22.7"

and W38°57'00.8") in March 2016. The material was identified by Dr. Maria Iracema Bezerra Loiola at the Herbário Prisco Bezerra (EAC), Departamento de Biologia, Universidade Federal do Ceará, Fortaleza, CE, Brazil, where the voucher specimens (No. 0056696) were deposited.[19]

Extraction and isolation of flavonoid

Leaves (100 g) of *L. camara* were ground to powder and extracted with ethanol (EtOH) at room temperature. The solvent was removed under reduced pressure to give an EtOH extract. The EtOH extract (35.49 g) was fractionated coarsely on a silica gel column by elution with hexane, EtOAc and MeOH. The ethyl acetate fraction resulted in the formation of a precipitate yielded bioside flavone named 5"-O-α-L-raminopyranosyl-1'-β-D-glycopyranosyl-4',6-dimethoxyflavone (Figure 1). The extraction, isolation and structural determination of the flavonoid used in this study were previously reporting in the literature.[19]

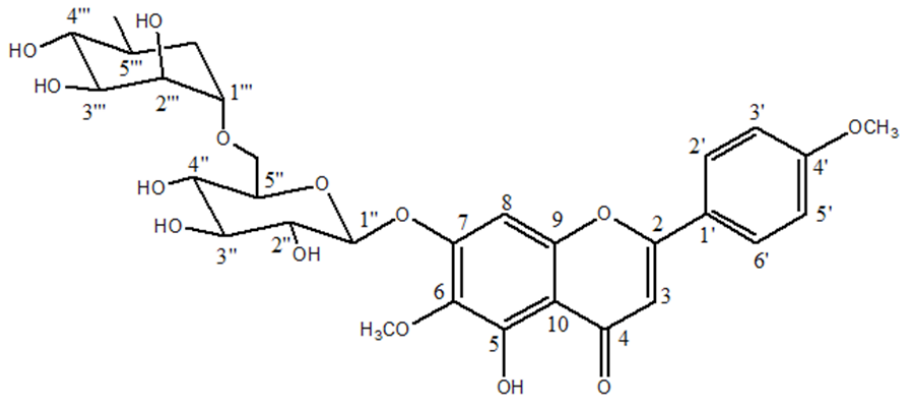

Figure 1 Molecular structure of the pectolinarin.

Microorganisms

The standard microorganisms used in the tests were obtained through the National Institute for Quality Control in Health (INCQS) of the Oswaldo Cruz Foundation, Ministry of Health, and the multi-resistant ones were obtained from the Federal University of Pernambuco- UFPB. The bacterial strains used were *Escherichia coli* (standard *E. coli* ATCC 10536 and multidrug-resistant *E. coli* 27) and *Staphylococcus aureus* (standard *S. aureus* ATCC 25923 and multiresistant *S. aureus* 358), with their resistance profiles being identified and reported in Table 1. All strains were maintained on Heart Infusion Agar (HIA, Difco Laboratories Ltda.). Prior to the assays, the strains were cultured for 24h at 37°C in Heart Infusion Agar (HIA, Difco Laboratories Ltda.).

Table 1 Pectolinarin microbiological data

	MIC	
Bacterial strain	Pectolinarin	
E. coli 27	≥ 1024µl/mL	
E. coli ATCC10536	≥ 1024µl/mL	
S. aureus 358	≥ 1024µl/mL	
S. aureus ATCC25923	≥ 1024µl/mL	
Modulation		
	***S. aureus* 358**	***E. coli* 27**
Gentamycin	40,32 µl/mL	40,32 µl/mL
Penicillin	512 µl/mL	≥ 1024 µl/mL
Norfloxacin	512 µl/mL	101,59 µl/mL
Gentamycin + EP	8 µl/mL	40,32 µl/mL
Penicillin + EP	≥ 1024 µl/mL	≥ 1024 µl/mL
Norfloxicin+ EP	512 µl/mL	128 µl/mL

Drugs

The antibiotics gentamicin (aminoglycoside), norfloxacin (fluoroquinolone), and penicillin (β-lactam) were used to evaluate the antibiotic potentiating activity of the pectolinarin flavonoid. All drugs were diluted in sterile water to concentration 5000 mg/mL. The solutions were prepared following the recommendations of the National Clinical Laboratory Standards Committee - NCCLS (NCCLS, 2003).

Antimicrobial activity

The MIC (minimum inhibitory concentration) was determined in a 96 well sterile microdilution assay[20] 100 µL of the inoculum suspension (10^5 CFU/mL^{-1}) were removed and added to a Brain Heart Infusion broth (10% BHI), thereafter 100 µL of this solution was added to each well, followed by 100 µL of the pectolinarin compound which was added to the first well, followed by serial (1:1) dilutions up to the penultimate well of the microplate. The final concentration of the sample ranged from 512 - 8 µg/mL. The MIC was recorded as the lowest concentration capable of inhibiting growth.

Antibiotic potentiating activity of the flavonoid pectolinarin

The pectolinarin compound was evaluated as a potentiator of antibiotic action against clinically isolated microorganisms. The MIC of the antibiotics was evaluated in the presence and absence of the test solution in sterile microplates. The antibiotics were evaluated at concentrations ranging from 2500 to 2.5 µg/mL. The test solution at subinhibitory concentrations (MIC/8) was mixed with 10% BHI broth and the inoculum suspensions. Then, 100 µL of the antibiotic was added to the first well of the plate, and serial dilutions (1:1) were performed up to the penultimate well of the microplate. The plates were incubated at 37 ºC for 24 hours, and after this period, the reading was observed using resazurin.

Statistical analysis

Test results were performed in triplicates and expressed as the geometric mean. Statistical analysis was performed using a one-way ANOVA followed by Tukey's post-hoc test, performed using the GraphPad Prism 6.02 software, considering significance with $p<0.05$.

Results and discussion

Antibacterial activity evaluation of the pectolinarin alone and in combination with antibiotics

The minimum inhibitory concentration (MIC) obtained for the flavone pectolinarin was ≥1024 µg/mL. Thus no differences were observed in the sensitivity of the pure natural substance against standard bacterial *S. aureus* and *E. coli* strains. However, the modulation by associating pectolinarin with gentamicin hears a change from resistant to sensitive with *S. aureus* 358 (Table 1).

The MIC determination of the antibiotics norfloxacin, gentamicin and penicillin in the presence and absence of the pectolinarin substance at a subinhibitory concentration (MIC/8=128 µg/mL) against multiresistant *S. aureus* 358 and *E. coli* 27 bacteria are shown in Figure 2.

In the antibiotic activity test against *S. aureus* 358, pectolinarin was seen to potentiate the inhibition of bacterial growth when associated with gentamicin, showing significant synergism with $p<0.0001$. An antagonistic effect was observed with the association of the compound with penicillin with a significance of $p<0.0001$, while non-significant results were obtained with the antibiotic norfloxacin. When *E. coli* 27 was the bacterial strain tested, no significant changes in MICs were observed with the association of pectolinarin with the tested antibiotics.

Aminoglycosides present bactericidal action against aerobic Gram-negative bacteria, some staphylococci, and *Mycobacterium tuberculosis*.[21] However, Gram-positive bacteria are more sensitive to antimicrobial action due to the presence of a bacterial wall that normally does not restrict the penetration of toxic molecules into the bacteria, whereas Gram-negative bacteria possess a barrier system consisting of an outer membrane with a cell wall formed by phospholipids, lipopolysaccharides and proteins making it impermeable to antibacterial agents, resulting in a greater resistance of these bacteria to antibiotics.[22,23]

In vitro studies have shown that pectolinarin possesses antiviral activity against the herpes simplex HSV-1 and dengue DENV-2 virus,[24] anti-inflammatory and anti-allergic actions,[25] as well as presenting an effective hepatoprotective action in a rat model of liver damage caused by D-galactosamine.[26]

Antimicrobial resistance has become a major public health problem affecting all countries,[27,28] where antimicrobial resistance may be a natural phenomenon developed by the pathogenic organism itself. However, the poor use of medications accelerates this process increasing resistance levels (WHO 2005).[29]

Treating various types of infectious diseases has become difficult, mainly due to the low efficiency of antimicrobials against today's bacteria,[30,31] which reinforces the need for the validation of new plant-derived drugs through research that will be useful in combating the spread of pathogenic microorganisms.[32–34]

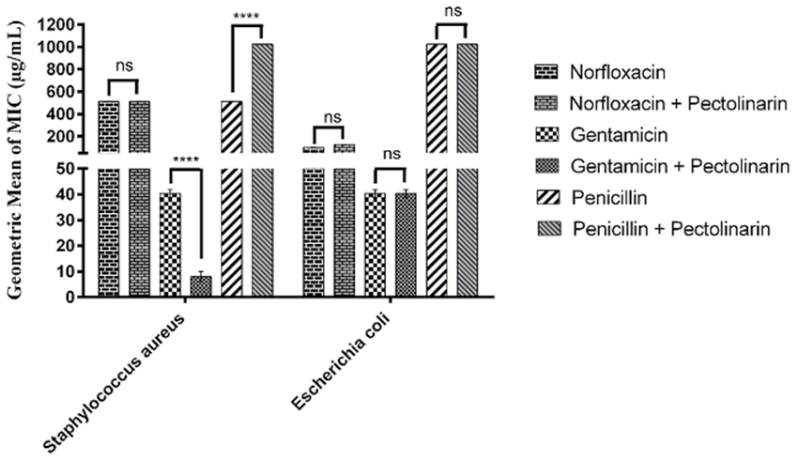

Figure 2 MIC of the antibiotics norfloxacine, gentamicin, and pencilillin the presence and absence of the pectolinarin in concentration MIC/8 (128µg/mL), towards *S. aureus* 358 and *E. coli* 27. Statisically significant values with *** p<0.0001- Non significant values (ns) with p>0.05.

Conclusion

The pectolinarin flavonoid when combined with the gentamicin antibiotic potentiated its action Gram-positive *S. aureus* bacteria. Moreover, an antagonistic effect was observed when the pectolinarin was combined with the penicillin antibiotic against the multiresistant *S. aureus* 358 strain. This research suggests that pectolinarin is a compound with potential application as an antibacterial drug.

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Conflicts of interest

The authors declare no conflict of interest.

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