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Original Article

Virtual screening of secondary metabolites of the genus *Solanum* with potential antimicrobial activity

Renata Priscila Costa Barros[a], Emidio Vasconcelos Leitão da Cunha[b], Raïssa Mayer Ramalho Catão[b], Luciana Scotti[a], Maria Sallett Rocha Souza[a], Amanda Amona Queiroz Brás[a], Marcus Tullius Scotti[a,*]

[a] *Laboratorio de Quimioinformática, Programa de Pós-graduação em Produtos Naturais e Sintéticos Bioativos, Universidade Federal da Paraíba, João Pessoa, PB, Brazil*
[b] *Departamento de Farmácia, Universidade Estadual da Paraíba, Campina Grande, PB, Brazil*

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ABSTRACT

Infectious diseases are a health problem today and have high mortality rates with a wide diversity of potentially pathogenic microorganisms. Research that is based either on the search for new drugs from plants or on the improvement of phytotherapeutics is prominent and continues to play an important role nowadays. From this perspective, use of *in silico* studies to carry out investigations of new molecules potentially active for methicillin-resistant *Staphylococcus aureus* and *Escherichia coli* using an in-house database with 421 different secondary metabolites selected from the literature from *Solanum* genus was performed. We also realized an *in vitro* study with strains of *S. aureus* and *E. coli* and compared the results. Two databases from ChEMBL were selected, the first one with activity against methicillin-resistant *S. aureus* and another against *E. coli*. The compounds were classified according to the pIC_{50} values to generate and validate the model using a "Random Forest". The "Random Forest" prediction model for methicillin-resistant *S. aureus* obtained an accuracy of 81%, area under the Receiver Operating Characteristic curve of 0.885, selecting eight molecules with an active potential above 60%. The prediction model for *E. coli* obtained an accuracy rate of 88%, area under the Receiver Operating Characteristic curve of 0.932, selecting four molecules with potential probability above 84%. Rutin proved to be potentially active in the *in silico* study for *S. aureus* and *E. coli*. Microbiological tests have shown that rutin has activity only for *E. coli*. An interaction study with strains of *S. aureus* ATCC 25923, a standard strain sensitive to all antibiotics, and SAM-01, a multidrug-resistant strain, was designed. There was interaction only between rutin and oxacillin, one of the three antibiotics studied in the interaction, for the strain SAM-01, reducing the resistance of this strain.

Introduction

Research that is based on the search for new medicines from plants or in the improvement of already existing phytotherapy has been prominent and continues to play an important role these days. In accordance with research realized by Newman and Cragg (2016) between the years 1981 and 2014, the area of natural products still produces or is involved in roughly 50.6% of all new drugs approved by the FDA and similar organizations.

Solanum is the biggest genus of the Solanaceae family, with 1500 species and roughly 5000 epithets described (Melo et al., 2011; Vorontsova and Knapp, 2012). The genus has a wide distribution in the world; Brazil is represented by 260 species, of which 127 are endemic (Agra et al., 2009).

The *Solanum* genus presents great wealth and diversity of properties; between them, they show the ability to biosynthesize steroids and free or glycosylated alkaloids, flavonoids, that are of interest therapeutically, which present a big variety of pharmacological activities, like cytotoxic activity, anticancer, anti-inflammatory, antiulcerogenic and antimicrobial (Pinto et al., 2011; Ordaz et al., 2011).

Infectious diseases are a world health problem today and have high mortality rates. There is a wide diversity of potentially pathogenic microorganisms, *including Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa*, and their ability to become resistant to the antibiotics available on the market reduces the chances of infection prevention and control (Ventola, 2015; Das et al., 2016).

Currently, there is a series of tools for studying the effects of diverse substances on an organism. The techniques of virtual screening represent a major advance in drug planning today, through the use of *in silico* methods, large banks of molecules are

* Corresponding author.
E-mail: mtscotti@ccae.ufpb.br (M.T. Scotti).

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automatically analyzed. Beyond identification of new potentially active molecules, the virtual screening also aims at the removal of molecules identified as toxic or having unfavorable pharmacokinetic and pharmacodynamic properties (Song et al., 2009; Prado-Prado et al., 2007, 2009; Speck-Planche and Cordeiro, 2014; Kleandrova et al., 2016).

In this study, was realized a bibliographic survey of secondary metabolites isolated from the *Solanum* genus, creating a bank of molecules. From the molecule bank created, two prediction models of molecules potentially active against pathogenic bacterium methicillin-resistant *S. aureus* (MRSA) and *E. coli* were generated. In addition, a study *in vitro* with strains of these bacterial species was realized and the results compared.

Experimental

Dataset

From the ChEMBL database, we selected two sets of chemical structures for construction of two predictive models. The first set had 1032 diverse chemical structures that had been studied (*in vitro*) and had inhibited strains of *S. aureus*. The compounds were classified using values of $-\log IC_{50}$ (mol/l) = pIC_{50}, which led us to assign 470 actives ($pIC_{50} \geq 5$) and 562 inactives ($pIC_{50} < 5$). In this case, IC_{50} represented the concentration required for 50% inhibition of strains of *S. aureus.*

The second set of chemical structures was composed of 1325 molecules that had inhibited strains of *E. coli*. The compounds were also classified using the value of pIC_{50}, which led us to assign 777 actives ($pIC_{50} \geq 4.6$) and 548 inactives ($pIC_{50} < 4.6$). The classification of pIC_{50} was different from the set of *S. aureus* because it was attempted to divide the amounts of active and inactive molecules close to half.

Another dataset of molecules isolated from the *Solanum* genus was built from a bibliographic survey in the research base Web of Science, covering a total of 550 published papers between the years 2016 and 1991. In this bank, 421 chemical structures of different classes of secondary metabolites (highlighting steroids and steroidal alkaloids) of many species of the *Solanum* genus were cataloged (Scotti et al., 2018).

For all structures, Smiles codes were used as input data in a Marvin 14.9.1.0, 2014, ChemAxon (http://www.chemaxon.com). We used Standardizer software [JChem 14.9.1.0, 2014; ChemAxon (http://www.chemaxon.com)] to canonize structures, add hydrogens, perform aromatic form conversions, clean the molecular graph in three dimensions, and save compounds in sdf format.

Three-dimensional (3-D) structures were used as input data in the software Dragon 7.0 (Kode, 2016) to generate descriptors, where it is possible to predict the biological and physicochemical properties of the molecules. This calculus was realized for both sets of chemical structures with knowledge of the activity for MRSA and *E. coli.*

Prediction model

The Knime 3.4.0 software (Knime 3.4.0 the Konstanz Information Miner Copyright, 2003–2014, www.knime.org) was used to perform all the following analyses. The descriptors and class variables were imported from the software Dragon 7.0, and for each one the data were divided using the "Partitioning" node with the "stratified sample" option to create a training set and a test set, encompassing 80% and 20% of the compounds, respectively. Although the compounds were selected randomly, the same proportion of active and inactive samples was maintained in both sets. However, for the *E. coli* model, the data were divided into 70% for the training set and 30% for the test set; due to the greater number of molecules in the *E. coli* bank it was possible to put more molecules into the training set.

For internal validation, we employed cross-validation using ten randomly selected, stratified groups, and the distributions according to activity class variables were found to be maintained in all validation groups and in the training set. Descriptors were selected, and a model was generated using the training set and the Random Forest algorithm (RF) (Salzberg, 1994), using the WEKA nodes (Hall et al., 2009). The parameters selected for RF included the following settings: number of trees to build = 100, seed for random number generator = 1, for the *S. aureus* model, and 50 the number of trees to build and two seeds for the *E. coli* model.

The internal and external performances of the selected models were analyzed for sensitivity (true positive rate, *i.e.*, active rate), specificity (true negative rate, *i.e.*, inactive rate), and accuracy (overall predictability). In addition, the sensitivity and specificity of the Receiver Operating Characteristic (ROC) curve were found to describe true performance with more clarity than accuracy.

Biological activity

For the antimicrobial activity screening, one clinical strain of *S. aureus* was used, SAM-01 (MRSA), belonging to the Laboratorio de Microbiologia da Universidade Estadual da Paraíba, and two reference strains, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. For the inoculum preparation, isolated colonies of new cultures (24 h) were selected, and with the aid of an inoculation loop, they were transferred to a tube containing 5 ml of 0.85% NaCl. They were homogenized, comparing their turbidity with a 0.5 tube of the McFarland scale (1.5×10^8 UFC/ml).

Antibiograms were carried out by disk diffusion in a solid medium according to CLSI, 2010, for the determination of the sensitivity profile of strain SAM-01.

For determination of the antimicrobial activity and the minimum inhibitory concentration (MIC), sterile microplates were used containing 96 wells with flat bottoms, where 100 μl of Brain Heart Infusion (BHI) broth was poured into each well. Rutin was used in a concentration of 6674 μM and 100 μl transferred to the first well. Dilutions were then performed to obtain concentrations between 10 and 6.55 μM. BHI broth with inoculum was used as the positive control and just the BHI broth was used as the negative control. In addition, the solvent control used for the dissolution of the products, DMSO, was inserted.

The experiment was realized with rutin diluted in distilled water and 5% v/v aqueous solution of DMSO, which in the first well became 2.5%. 10 μl of the inoculum in that concentration was also dispensed 1.5×10^8 CFU/ml. The plates were incubated at 35–37 °C for 24 h, and the experiments were performed in triplicate.

Bacterial viability was detected by adding 20 μl of resazurin (0.01%) in aqueous solution. The plates were reincubated at 37 °C for 2 h, and in those wells where bacterial growth occurred the resazurin changed to pink. MIC was defined as the lowest concentration of antibacterial agents that inhibited visible growth, as indicated by resazurin staining.

The analysis of rutin interference over the effectiveness of conventional antibiotics, oxacillin, penicillin, and amoxicillin + Ac was performed. Clavulanic, for the strains of *S. aureus*, was performed by disk diffusion. For the interaction test, 50 μl of rutin at a concentration of 6674 μM, *i.e.*, 2048 μg/ml, was added to each antibiotic used as well as to sterile disks to observe comparatively whether the addition of the product caused some change in the size of the inhibition halos. The disks of each antibiotic were also inserted in the plate for visualization of the sensitivity profile of the strains and the occurrence of synergism or antagonism with rutin use. Sterile

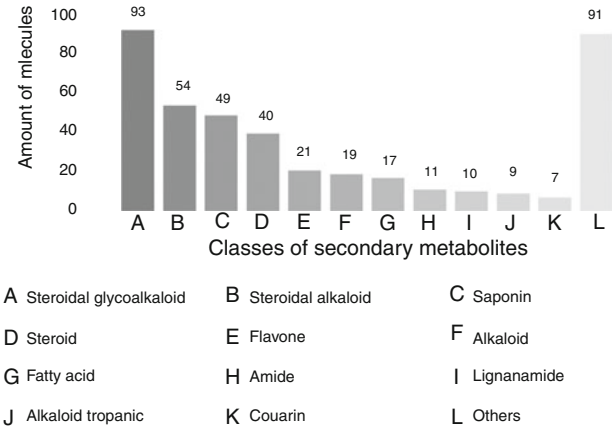

Fig. 1. Classes and subclasses of metabolites isolated from the *Solanum* genus.

disks were further filled with the diluent used to dilute rutin, 2.5% DMSO and sterile distilled water.

It was regarded as an interactive effect when there was a change in diameter of the inhibition zones (halo) of microbial growth after this process, a synergistic interactive effect if the antibacterial showed an increase of ≥2 mm when compared with the inhibition zones formed by the tested alone. If it showed a smaller diameter than the one formed by isolated activity, an antagonistic effect was considered. These tests were performed in triplicate and the results were obtained by the mean of the inhibition zones formed (Oliveira, 2006).

Results and discussion

The secondary metabolites' dataset was composed of 734 molecules, with 421 different chemical structures, from 110 species of the *Solanum* genus. These 421 structures were classified into 54 classes of secondary metabolites, highlighting: steroidal glycoalkaloid (93), steroidal alkaloid (54), saponin (49), steroid (40), flavones (21), among others represented in Fig. 1. This dataset is available in Sistemat X (Scotti et al., 2018).

In accord with these results, it is possible to observe that the main classes are the steroidal glycoalkaloids, alkaloids, saponin, steroids, and flavones, which are considered chemotaxonomic markers of this genus (Pereira et al., 2016).

The Dragon 7.0 (Kode, 2016) software generated 1232 descriptors for 1033 molecules of known activity against MRSA. These descriptors were used as input data to the Knime software for generation of the predictive model.

The Dragon 7.0 (Kode, 2016) calculation gave roughly 5270 molecular descriptors covering most of the theoretical approaches. These descriptors are organized into thirty logical blocks. The descriptors' list includes the simplest types of atoms, functional groups and fragment counting, topological and geometric descriptors, three-dimensional descriptors, but also several estimates of properties such as log *P* and Lipinski (Kode, 2016).

The molecular descriptors with the information of qualitative biological activity were used for model generation with the RF machine learning. The *S. aureus* model with molecules with $pIC_{50} \geq 5$ were considered as active, with a total of 470 molecules, and the molecules with $pIC_{50} < 5$ were considered as inactive, totaling 562 molecules. For the *E. coli* model, the molecules with $pIC_{50} \geq 4.6$ were considered as active, with a total of 777 molecules, and the molecules with $pIC_{50} < 4.6$ were considered as inactive, totaling 548 molecules.

Analyzing the *S. aureus* model, you can see that the cross-validation and the test demonstrated similar statistical performance, with hit rates higher than 74%, while the *E. coli* model had hit rates higher than 83%. The training had an almost perfect performance, on both models, with hit rates of 99%, as can be seen in Tables 1 and 2, which summarize the statistical rates of the RF model.

For the training and test, the *S. aureus* RF model had similar rates for the active and inactive molecules (99% and 81%, respectively), but for the cross-validation, there was a higher hit rate for the inactive compounds, 78%, while the hit rate for active compounds was 74%. In addition, for the cross-validation and test, the *E. coli* RF model had similar rates for the active molecules greater than 88%, but for the inactive, they were slightly smaller, 74% cross-validation and 80% in the test.

With these results, it was possible to calculate the Matthews correlation coefficient (MCC) for general evaluation of the two models. The MCC correlates the observed and predicted binary classifications, resulting in a value between −1 and +1, where +1 is a perfect prediction and −1 indicates a complete disagreement between prediction and observation. For the test, the value obtained for the *S. aureus* model was 0.68 and 0.62 in the cross-validation, and for the *E. coli* model was 0.71 in the test set and 0.70 in the cross-validation, revealing the good prediction of the two models.

The ROC graph that analyzes the model performance was generated for the test set for the two models and the area under the curve obtained for the *S. aureus* model was 0.885, Fig. 2. The area under the curve obtained for the *E. coli* model was 0.9329, Fig. 3. Knowing that a perfect model has an area under the curve equal to 1, it is possible to state that the models above are capable of performing a high classification rate for this RF method.

The two models were used to triage the secondary metabolites' bank of the *Solanum* genus for investigating possible bioactive molecules against MRSA and *E. coli*. Molecules with probability greater than 50%, pIC50 ≥ 5 for the *S. aureus* model and pIC50 ≥ 4.5 for the *E. coli* model, were considered active, totaling 30 molecules selected by the *S. aureus* model and 221 molecules selected by the *E. coli* model.

On the *S. aureus* model, molecules with probability greater than 60% were selected for this study, to increase the restriction, totaling eight potentially active molecules against MRSA. Table 3 shows the molecules selected and their respective classes of secondary metabolites and species from which they were isolated.

Of the 221 active molecules on the *E. coli* model, 26 are likely to be active between 80 and 88%, 77 with potential activity between 70 and 79% probability, 64 molecule with a probability of activity between 60 and 69%, and finally, 54 molecules between 50 and 59% of active potential. The molecules with the highest active potential, of 84–88% probability,

Table 1
Summary of cross-validation and test for the chemical compounds of the activity known as methicillin-resistant *Staphylococcus aureus* (MRSA) using the Random Forest model.

	Cross validation			Test		
	Sample	Predict	%Hit	Sample	Predict	%Hit rate
Active	376	280	74	94	77	81
Inactive	410	322	78	102	82	80
General	786	602	76	196	159	81

Table 2
Summary of cross-validation and test for chemical compounds of known activity for multidrug-resistant *Escherichia coli* using the Random Forest model.

	Cross validation			Test		
	Sample	Predict	%Hit	Sample	Predict	%Hit rate
Active	544	486	89	233	206	88
Inactive	383	285	74	165	127	80
General	927	771	83	398	398	83

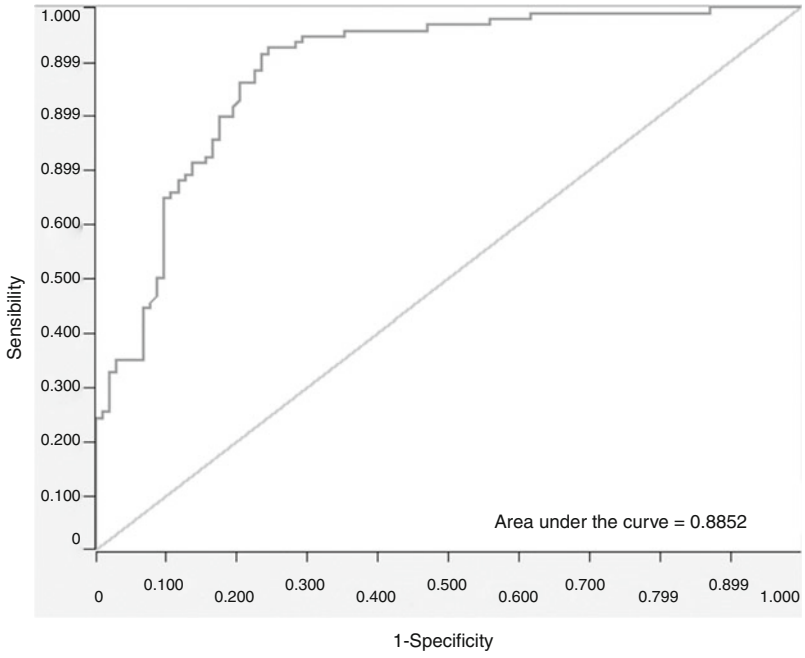

Fig. 2. ROC plot with the area under a curve for the MRSA model test set obtained with Random Forest.

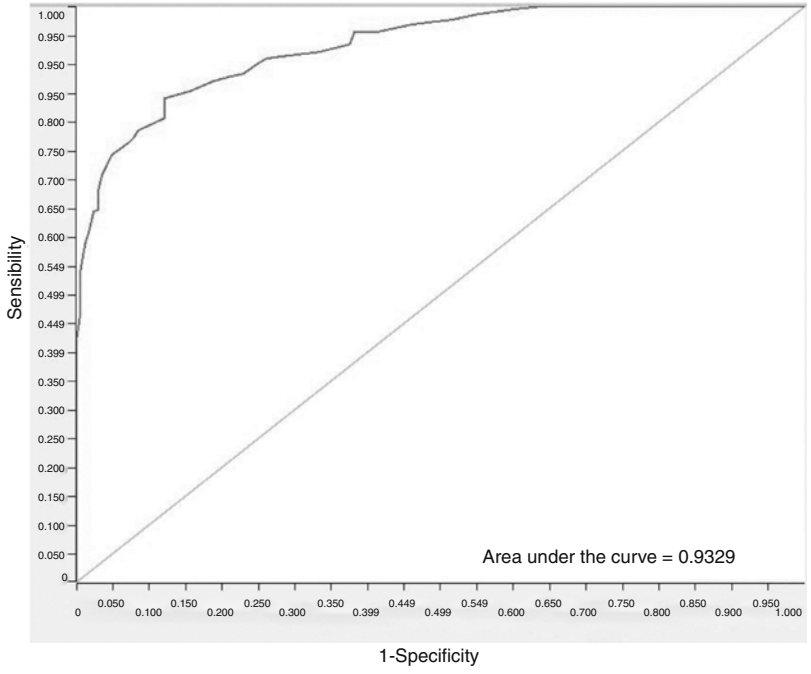

Fig. 3. ROC plot with the area under the curve for the test set of the *E. coli* model obtained with Random Forest.

Table 3
Molecules selected with higher active potential, on the *Staphylococcus aureus* model, and their respective classes of secondary metabolites and species from which they were isolated.

Chemical structures	Class	Species
Melongenamide D	Lignanamide	*Solanum melongena*
Grossamide	Lignanamide	*Solanum melongena*
N-cis-Grossamide	Lignanamide	*Solanum tuberosum*
N-trans-Grossamide	Lignanamide	*Solanum tuberosum*
Melongenamide B	Lignanamide	*Solanum melongena*
Tiliroside	Flavone	*Solanum crinitum*
Melongenamide A	Lignanamide	*Solanum melongena*
Cannamisin A	Lignanamide	*Solanum melongena*
Rutin	Flavone	*Solanum lycopersicum*

Table 4
Molecules selected with higher active potential, on the *Escherichia coli* model, and their respective classes of secondary metabolites and species from which they were isolated.

Chemical structures	Class	Species
Abutiloside J	Glycosteroid	*Solanum abutiloside*
Abutiloside A	Glycosteroid	*Solanum abutiloside*
Solasodoside E	Glycosteroid	*Solanum sodomaeum* L.
Abutiloside R	Glycosteroid	*Solanum abutiloside*
Rutin	Flavone	*Solanum lycopersicum*

are available in Table 4, as well as the class of secondary metabolites to which they belong and the species from which these molecules have been isolated and reported in the literature.

The rutin molecule is also present in Tables 3 and 4; it has 53% and 56% probability of being potentially active on the *S. aureus* and *E. coli* models, respectively, and it was chosen for the *in vitro* studies because of its availability.

Rutin diluted in sterile distilled water or in 2.5% DMSO showed no activity against the two strains of *S. aureus*, ATCC 25923 and SAM-01. However, for the *E. coli* standard strain, ATCC 25922, rutin exhibited activity when diluted in 2.5% DMSO with MIC value of 455 μM (256 μg/ml). These results can be seen in Fig. 4.

Souza (2009) and Oliveira (2014) found similar results when evaluating rutin's antimicrobial effect (Sigma-Aldrich) against *Salmonella enterica*, *E. coli*, *S. aureus*, and *P. aeruginosa*. In these studies, different concentrations between 15.625 μg/ml and 10000 μg/ml and different ways of solubilizing rutin, e.g., in methanol, distilled water, 3% DMSO, were used.

Those results differ from that of this research regarding rutin activity for *E. coli*. These differences in results can be attributed to the way it was diluted, as well as the product brand (rutin).

Rutin is a glycosidic flavonol and has great therapeutic importance for improving the resistance and permeability of capillary vessels, with antioxidant activities, anti-inflammatory, anticarcinogenic properties, among others (Brecho et al., 2009). According to Martini et al. (2009), rutin has an activity for some Gram-positive and Gram-negative bacteria.

Rutin showed activity against *E. coli*, corroborating with the result obtained in the predictive model of *E. coli* where rutin had 56% probability of active potential. However, in the predictive model of *S. aureus*, rutin had an active potential of 53%, which disagrees with the *in vitro* results because rutin showed no activity for any of the *S. aureus*, ATCC 25923 and SAM-01 strains.

A fact to be observed is that for the generation of the models, the IC_{50} was considered, and in the *in vitro* tests, MIC was considered.

A study was also carried out of the interaction between rutin and three of the antibiotics that the MRSA strain, SAM-01, is resistant to, namely, oxacillin (which is equivalent to methicillin), amoxicillin + clavulanic acid, and penicillin, to observe if rutin has the capacity to interact with some of these antimicrobials and alter the final response in the bacterium.

Only rutin diluted in sterile distilled water presented an interactive effect for one the MRSA strains, SAM-01. This strain is resistant to oxacillin, there is no inhibition halo formation; when evaluating the effect of rutin on it, a 14 mm inhibition halo was formed (Fig. 5). The presence of this halo, according to the sensitivity values in the literature, is not sufficient to make the strain sensitive to the action of this interaction, but it was enough to decrease the resistance of this strain to oxacillin.

Conclusion

Through the *in silico* tools used in this work, it was possible to generate models to trace virtually the database of the *Solanum* genus. The *S. aureus* model selected thirty molecules with potential effect against MRSA, where eight molecules have a probability greater than 60%. With the *E. coli* model, it was possible to

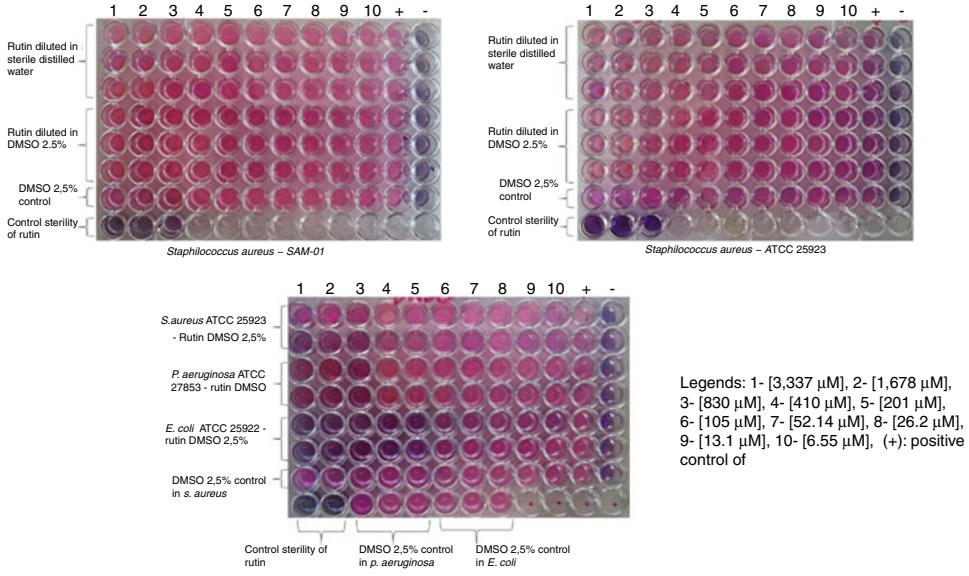

Fig. 4. Analysis of the antimicrobial activity of rutin solution (diluted in 2.5% DMSO and another solution diluted in sterile distilled water) for *S. aureus* strain ATCC 25923 and SAM-01 and *E. coli* ATCC 25922.

Fig. 5. Interactive effect of rutin solution, diluted in sterile distilled water, with the antibiotics oxacillin, amoxicillin + ac. clavulanic acid, and penicillin, against the strain SAM-01 by means of the disk diffusion technique. First line – sterile disk imbibed with rutin solution; from left to right we have: second line – oxacillin disk (OXI), OXI disk soaked with rutin solution, OXI disk soaked with sterile distilled water; third line – penicillin disk (PEN), PEN disk imbibed with rutin solution, PEN disk soaked with sterile distilled water; fourth line – amoxicillin + ac. clavulanic acid (AMC), AMC disk soaked with rutin solution, AMC disk soaked in sterile distilled water.

identify 221 molecules potentially active for this bacterium from the database of the *Solanum* genus. Among these molecules, 26 molecules had an active potential between 80 and 88% probability. The *in vitro* tests performed revealed that rutin had no activity for *S. aureus* strains, being active just for the *E. coli* strain. Rutin was able to interact with the oxacillin antibiotic in the SAM-01 (MRSA) strain, being able to reduce the resistance of this bacterium to this antibiotic.

Conflicts of interest

The authors declare no conflicts of interest.

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