COMPARATIVE EVALUATION OF ANTIBIOTIC AND ANTIBIOTIC MODIFYING ACTIVITY OF QUERCETIN AND ISOQUERCETIN IN VITRO

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ABSTRACT: The use of secondary metabolites with biological properties, such as flavonoids, has been increasingly documented. This work evaluates the in vitro antibiotic activity of two flavonoids: quercetin and isoquercetin. The microdilution broth test was used to measure antimicrobial activity against standard and multiresistant strains of E. coli and S. aureus and strains of Candida. These flavonoids were also tested for a modulatory effect on aminoglycoside antibiotics. The flavonoids studied did not show significant antibacterial activity, while only isoquercetin demonstrated notable antifungal activity, inhibiting the growth of Candida krusei at a concentration of 32 μg/mL. In relation to modifying activity, there was no potentiation of the antibiotics tested. Isoquercetin showed antagonism with all the aminoglycosides examined, considerably increasing their minimal inhibitory concentrations (MICs). In light of these results, more in-depth studies are necessary, aimed at standardizing the protocols of antibiotic assays, as well as evaluating the effect of test substances against other classes of antimicrobials.

KEY WORDS: Antibiotic activity, Flavonoids, Isoquercetin, Quercetin

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INTRODUCTION

In the last decades, there has been a growth in the incidence of adverse effects associated with conventional drugs, together with an increase in microbial resistance to antibiotics (Paulo et al., 1992). Drug development research continues to search for new natural products that show potential efficacy in the treatment of various microbial infections. Therefore, the study of natural products of plant origin with antimicrobial activity has taken on broad perspectives (Gutierrez et al., 2008). The antimicrobial activity shown by plant or food - derived drugs can be due to the presence of flavonoids, tannins, alkaloids, saponins and terpenes (Veluri et al., 2004; Kuete et al., 2006). Although widely recognized for their antifungal action, the flavonoids also possess considerable antibacterial and antiprotozoal activity (Marcucci, 1996). Some investigators have demonstrated their importance as a source of new antimicrobial agents (Zampini et al., 2005).

Various therapeutic proprieties of the flavonoids have been studied, notably their antioxidant and anticarcinogenic potential and their protective effects toward renal, cardiovascular and hepatic systems, such as quercetin (Figure 1), the principal flavonoid in the human diet (Behling et al., 2004). Isoquercetin (Figure 2) is a glycosylated flavonoid derived from quercetin. Some studies have reported their cytotoxic, phytotoxic, antimicrobial and antioxidant potential (Razavi et al., 2009), and even antinoceptive activity (Gadotti et al., 2005). High concentrations of isoquercetin and other compounds derived from quercetin are present in apples, tea and onion, which could reduce the symptoms of fatigue, depression, anxiety, heart disease and cancer (Ribeiro et al., 2001). In the present study, we evaluated the antibacterial and antifungal activity of the flavonoids quercetin and isoquercetin and their antibiotic modifying activity when combined with aminoglycoside antibiotics...
against multiresistant strains of *Staphylococcus aureus* and *Escherichia coli*.

**TABLE 1. Bacterial source and antibiotic resistance profile.** Ast, Aztreonam; Ax, Amoxicillin; Amp, Ampicillin; Ami, Amikacin; Amox, Amoxicillin; Ca, Cefadroxil; Cf, Cefaclor; Cf, Cefalotin; Caz, Cefazidime; Cip, Ciprofloxacín; Chlo, Chloramphenicol; Im, Imipenem; Kan, Kanamycin; Szt, Sulfameton; Tet, Tetracyclin; Tob, Tobramycin; Oxa, Oxacillin; Gen, Gentamicin; Neo, Neomycin; Para, Paramomycin; But, Butirosin; Sis, Sisomicin; Net, Netilmicin.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Source</th>
<th>Antibiotic resistance</th>
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<tbody>
<tr>
<td><em>E. coli</em> 27</td>
<td>Surgical wound</td>
<td>Ast, Ax, Amp, Ami, Amox, Ca, Cf, Cf, Caz, Cip, Clo, Im, Can Szt, Tet, Tob.</td>
</tr>
<tr>
<td><em>S. aureus</em> 358</td>
<td>Surgical wound</td>
<td>Oxa, Gen, Tob, Ami, Can, Neo, Para, But, Sis, Net</td>
</tr>
</tbody>
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**TABLE 2. MIC values (µg/mL) of quercetin and isoquercetin in the absence and presence of aminoglycoside antibiotics.**

<table>
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<tr>
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</thead>
<tbody>
<tr>
<td>Isoquercetin</td>
<td>128.0</td>
<td>128.0</td>
<td>-</td>
<td>128.0</td>
<td>128.0</td>
<td>-</td>
</tr>
<tr>
<td>Quercetin</td>
<td>128.0</td>
<td>128.0</td>
<td>-</td>
<td>128.0</td>
<td>128.0</td>
<td>-</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>156.3</td>
<td>39.1</td>
<td>1250.0</td>
<td>312.5</td>
<td>312.5</td>
<td>312.5</td>
</tr>
<tr>
<td>Amikacin</td>
<td>156.3</td>
<td>156.3</td>
<td>625.0</td>
<td>312.5</td>
<td>312.5</td>
<td>312.5</td>
</tr>
<tr>
<td>Neomycin</td>
<td>39.1</td>
<td>39.1</td>
<td>156.3</td>
<td>39.1</td>
<td>39.1</td>
<td>39.1</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>9.8</td>
<td>9.8</td>
<td>78.1</td>
<td>9.8</td>
<td>9.8</td>
<td>9.8</td>
</tr>
</tbody>
</table>

**MATERIALS AND METHODS**

Neomycin, kanamycin, gentamicin and amikacin were obtained from Sigma Chemical Corp., St. Louis, MO, USA. All drugs were dissolved in sterile water. Four bacterial strains were utilized: *Escherichia coli*, ATCC 10536 and clinical isolate EC27; *Staphylococcus aureus*, ATCC 25923 and clinical isolate SA358. We used two yeast strains: *Candida albicans* ATCC 40227 and *Candida krusei* ATCC 6538. The standard strains were obtained through the Instituto Nacional de Controle de Qualidade em Saude (INCQS) of the Oswaldo Cruz Foundation, Ministry of Health and the clinical isolates, from the Federal University of Paraiba – UFPB (Table 1). Quercetin and isoquercetin were purchased from MERCK – Germany.

Stock solutions of the test substances were prepared by dissolving 10 mg of the compound in 1 mL of dimethylsulfoxide (DMSO- MERCK), thus starting with an initial concentration of 10 mg/mL. The resulting solution was then diluted to 1024 µg/mL, also in DMSO, since dilution in sterile water caused precipitation of the substances. A control was included with only solvent to determine the interference in the antibiotic assay with the microorganisms used, since the toxicity of DMSO at high concentrations has already been demonstrated (Javadpour et al., 1996).

The minimal inhibitory concentration (MIC) of the test substances (quercetin and isoquercetin) was determined by the microdilution assay in BHI broth with suspensions of 10^5 CFU/mL and drug concentrations varying from 1 to 1024 µg/mL (Javadpour et al., 1996). MIC was defined as the lowest concentration of drug at which no growth of microorganisms was observed. In the evaluation of the test substances for modulatory effect on antibiotic activity, the MIC of the antibiotics were determined in the presence of quercetin and isoquercetin at sub-inhibitory concentrations (MIC/8), and then the plates were incubated at 37 ºC for 24 h (Coutinho et al., 2010).
RESULTS

Table 2 shows the antibacterial activity data for the test compounds. The flavonoids quercetin and isoquercetin displayed no activity against the standard and multiresistant bacterial strains. In relation to the yeast strains used, antifungal activity was found only for the strain of *Candida kruzei*, showing a MIC of 32 µg/mL, where no such activity was seen against *Candida albicans*.

Quercetin and isoquercetin were tested to see if they showed modulatory activity against microbial resistance to antibiotics. Table 2 shows that the flavonoid quercetin did not affect the antibacterial activity of the aminoglycoside antibiotics, when tested in combination. With respect to the combination of isoquercetin with the aminoglycosides, the modulatory effect observed was antagonistic, where there was an increase in MIC for the antibiotics tested against the clinical isolate EC27. There was no modulatory activity with regard to the multiresistant strain of *S. aureus*.

DISCUSSION

Some investigators have reported synergism between flavonoids and conventional antibacterial agents against resistant strains, and others have examined whether the activity of flavonoids is bacteriostatic or bactericidal (Cushnie and Lamb, 2005). On the other hand, the lack of antibiotic activity found in the present work is very much at odds with other reports. According to Cushnie and Lamb (2005), the antibacterial activity of flavonoids has been increasingly documented. Many researchers are one step further, where they have isolated and identified the structures of flavonoids that possess antibacterial activity, or have determined the activity of commercially available flavonoids, such as quercetin, 3-O-methylquercetin and various glycosides of quercetin (Rauha et al., 2000; Basile et al., 2000; Arima and Danno, 2002).

In many published studies on flavonoids, assay results of antibacterial activity appear to be very conflicting. In a study conducted by Basile et al. (2000), these authors demonstrated that apigenin had no activity against *S. aureus*, at concentrations up to 128 µg/mL. In another study, Sato et al. (2000) demonstrated that the flavone inhibited the growth of 15 MRSA strains and another five strains of *S. aureus* at concentrations between 3.9 µg/mL and 15.6 µg/mL. These inconsistencies can be due to variations in technique in each assay (Ng et al., 1996; Kim et al., 1999).

The present study showed that the flavonoids quercetin and isoquercetin have no antibacterial activity at the clinically relevant concentrations tested. Isoquercetin demonstrated antifungal activity against *Candida kruzei*, displaying a MIC of 32 µg/mL. These findings concur with those of Souza (2009) and Razavi et al. (2009), who demonstrated that quercetin and isoquercetin do not exert antibiotic effects against Gram-negative or Gram-positive bacteria at the concentrations utilized.

The combined use of antibiotics with other substances can lead to various effects, such as the antagonism observed between aminoglycosides and isoquercetin in the present study. According to Granowitz and Brown (2008), the antagonistic effects of the combined use of antibiotics can be attributed to mutual chelation. Behling et al. (2004) reported that the antioxidant activity of flavonoids, such as quercetin, is due to their chelating proprieties. This effect possibly explains the reduction in activity of the aminoglycoside antibiotics in the presence of isoquercetin.

It is important to note that use of natural products associated with allopathic medicines has already been reported in the literature (Calvet-Mir et al., 2008; Vandebroek et al., 2008; Veiga Junior, 2008), and this concomitant use can cause the development of serious toxic effects, which could lead to death (Cordeiro et al., 2005). This combination can be a risk to health, as could be observed in our assays with isoquercetin, a flavonoid present in natural foods such as fruits, where when combined with antibiotics significantly diminished their efficacy.

Reports of Egert et al. (2008; 2009) evaluating healthy patients demonstrated that, after the ingestion of 150 mg of quercetin by two weeks, the median maximum of plasma concentration was 431nmol/L (corresponding to 130.162 µg/mL). Our study indicates was observed the absence of any interference of quercetin on the aminoglicosydes antibiotic activity using 128 µg/mL, almost the same value of the plasmatic concentration of quercetin. About the isoquercetin (quercetin 3-O-ß-D-glucoside), the glucose linked to the structure apparently promotes the antagonism between this flavonoid and the antibiotics against the multiresistant strain of *E. coli*, enhancing the MICs.

This is the first report of the evaluation of the drug modifying activity of the glycosylated flavonoid isoquercetin in combination with aminoglycoside antibiotics. More in-depth studies are needed with the aim of standardizing antimicrobial assay protocols, as well as evaluating the effect of test substances on other classes of antibiotics.

CONFLICT OF INTEREST DISCLOSURE

The authors have not conflict of interest to disclose.

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REFERENCES


