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**RESEARCH ARTICLE**

# *In vitro* interference of *Momordica charantia* in the resistance to aminoglycosides

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**Abstract**

In this study, the ethanol extract of *Momordica charantia* L. (EEMC) (Cucurbitaceae) was tested for its antimicrobial activity against strains of *Escherichia coli*. The growth of two *E. coli* strains tested was not inhibited by the extract. The MIC and MBC values were ≥ 1 mg/mL for both strains of *E. coli* used. A synergistic effect between this extract and aminoglycosides was demonstrated. Similarly, a synergistic effect was observed of chlorpromazine on kanamycin and amikacin, indicating the involvement of an efflux system in the resistance to these aminoglycosides. The checkerboard method with combinations of aminoglycosides and EEMC demonstrated synergism with kanamycin and an additive effect with amikacin and neomycin. It is therefore suggested that extracts from *M. charantia* could be used as a source of plant-derived natural products with resistance-modifying activity. This is the first report about the modifying antibiotic activity of *Momordica charantia*, constituting a new weapon against bacterial resistance to antibiotics, as with chlorpromazine.

**Keywords:** *Aminoglycosides; chlorpromazine; ethanol extract; modifying antibiotic activity;* Momordica charantia

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## Introduction

With a growing incidence of infections resistant to antibiotics, natural products from plants could be interesting alternatives (Lu et al., 2007; Mbwambo et al., 2007). Some plant extracts and phytochemicals are known to have antimicrobial properties, and can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to demonstrate such efficacy (Gibbons, 2004; Gurib-Fakim, 2006).

Many plant extracts or products have been evaluated not only for direct antimicrobial activity, but also as resistance-modifying agents (Luqman et al., 2007). Several chemical compounds, synthetic or from natural sources, such as the phenothiazines and natural products, show an indirect effect against many species of bacteria, by enhancing the activity of a specific antibiotic, reversing the natural resistance of specific bacteria to given antibiotics, promoting the elimination of plasmids from bacteria such as *Escherichia coli*, and inhibiting transport functions of the plasma membrane in regard to given antibiotics. The inhibition of plasma membrane-based efflux pumps has been observed as well (Jana & Deb, 2006; Smith et al., 2007). The enhancement of antibiotic activity or the reversal of antibiotic resistance by natural or synthetic non-conventional antibiotics affords the classification of these compounds as modifiers of antibiotic activity.

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*Momordica charantia* L. (Cucurbitaceae) is a climber known as bitter melon, which grows worldwide. Several flavonoids with pharmacological and biological activities have been identified in *M. charantia* (Grover & Yadav, 2004; Ansari et al., 2005; Das et al., 2006; Reyes et al., 2006). Additionally, the triterpenoid cucurbitan and the protein MAP30 have shown anti-human immunodeficiency virus (HIV) and insecticidal activities, respectively (Bourinbaiar & Lee-Huang, 1995; Mekuria et al., 2005).

Aminoglycosides are potent bactericidal antibiotics targeting the bacterial ribosome, and the increase in cases of bacterial resistance to aminoglycosides is widely recognized as a serious health threat (Jana & Deb, 2006). In *Escherichia coli*, the main mechanisms of resistance to aminoglycosides are active drug efflux and enzymatic inactivation (Smith et al., 2007).

In this work, we tested an ethanol extract of *Momordica charantia* and chlorpromazine as resistance-modifying agents in an aminoglycoside-resistant strain of *E. coli*.

## Materials and methods

### *Strains*

The experiments were performed with the clinical *Escherichia coli* isolate EC27, which is resistant to neomycin and gentamicin (low level) and to amikacin and kanamycin [see the complete resistance profile given by Coutinho et al. (2005)]. The EC-ATCC 8539 strain of *Escherichia coli* was used as a positive control. All strains were maintained on heart infusion agar slants (HIA; Difco), and prior to assays, the cells were grown overnight at 37°C in brain heart infusion (BHI; Difco).

### *Plant material*

Leaves of *Momordica charantia* were collected in the county of Crato, Ceará State, Brazil. The plant material was identified by the botanist Dr. Arlene Pessoa and a voucher specimen, number 703, was deposited at the Herbarium "Dárdano de Andrade Lima" of Universidade Regional do Cariri – URCA.

### *Preparation of the ethanol extract of* Momordica charantia *(EEMC)*

A quantity of 200 g of aerial parts was dried at room temperature and powdered. The powdered material was extracted by maceration using 1 L of 95% ethanol as solvent at room temperature, and the homogenate was allowed to stand for 72 h at room temperature. The extract was then filtered, and the filtrate concentrated under vacuum in a rotary evaporator (Brasileiro et al., 2006). For the tests, the dry extract residue was dissolved in dimethylsulfoxide (DMSO).

### *Drugs*

Chlorpromazine, gentamicin, kanamycin, amikacin, and neomycin were obtained from Sigma Chemical Co. All drugs were dissolved in sterile water.

### *Drug susceptibility test*

The minimum inhibitory concentration (MIC) of EEMA, antibiotics, and chlorpromazine (CPZ) was determined in BHI by the microdilution assay, using suspensions of $10^5$ CFU/mL and a drug concentration range of 1024–1 μg/mL (two-fold serial dilutions) (Javadpour et al., 1996). MIC was defined as the lowest concentration at which no growth was observed. For the evaluation of EEMA as a modulator of antibiotic resistance, MICs of the antibiotics were determined in the presence of EEMA (128 μg/mL) and CPZ (16 μg/mL) at sub-inhibitory concentrations, and the plates were incubated for 24 h at 37°C. CPZ was used as a positive control for efflux pump inhibition.

### *Checkerboard method*

The EC27 strain was tested by the microdilution checkerboard technique (Eliopoulos & Moellering, 1991). Suspensions of $10^5$ CFU/mL of bacterial culture were prepared and distributed into microtiter plates containing various concentrations of the different drugs. The inoculated plates were incubated at 37°C for 24 h, and then evaluated for bacterial growth. In order to determine the activity of drug combinations, fractional inhibitory concentration (FIC) indices were calculated as $FIC^A + FIC^B$, where $FIC^A$ and $FIC^B$ represent the minimum concentrations that inhibited bacterial growth for drugs A and B, respectively: $FIC^A = MIC^A$ combination/$MIC^A$ alone and $FIC^B = MIC^B$ combination/$MIC^B$ alone. A mean FIC index was calculated based on the following equation: FIC index = $FIC^A + FIC^B$, and the interpretation made as follows: synergistic (< 0.5), additive (0.5–1.0), indifferent (> 1), or antagonistic (> 4.0).

## Results and discussion

The EEMC did not show a substantial antibacterial activity against either *E. coli* strain at 1024 μg/ml (MIC > 1024 μg/mL), which is consistent with other reports (Martinez et al., 1996; Rodríguez et al., 2006). However, many authors claim that *M. charantia* has antibacterial activity against other bacterial species (Grover & Yadav, 2004; Lans, 2007).

Although EEMC did not show appreciable antibacterial activity (Gibbons, 2004; Houghton et al., 2007), the addition of EEMA to the growth medium at 128 μg/mL (≤ 1/16 MIC) produced a dramatic reduction in the MIC for all aminoglycosides in the strain *E. coli* 27 (but not with ATCC 8539, possibly due the absence of any resistance mechanisms to aminoglycosides), demonstrating a synergistic effect of EEMC with aminoglycosides (Table 1).

Studies on interactions of natural products from *Momordica charantia* have been conducted for antidiabetic (Tongia et al., 2004) and anti-HIV drugs (Bourinbaiar & Lee-Huang, 1995), but as far as we know, natural products of *Momordica charantia* having a synergistic effect with aminoglycosides have not been previously reported.

An MIC reduction for kanamycin, neomycin, and amikacin was also observed when CPZ was added to the growth medium at 16 μg/mL (1/4 MIC), which indicates the involvement of an efflux pump in the resistance to these antibiotics (Table 1). The effect was also observed when EEMC was added to the medium, suggesting that EEMC is a putative inhibitor of an efflux pump (Table 1), but additional experiments are needed to confirm such mechanisms.

Phenothiazines, such as chlorpromazine, probably act on the plasma membrane of bacteria, affecting the efflux pumps (Salih et al., 1991; Reddy et al., 1992; Kristiansen & Amaral, 1997). This modification of drug permeability could enhance the activity of antibiotics that act within the cell, such as the aminoglycosides.

Efflux pumps have been known as resistance mechanisms of *E. coli* since the 1980s; they belong to the resistance-nodulation-cell division (RND) family of transporters and represent an important mechanism of multidrug resistance (MDR) that accounts for the resistance to aminoglycosides (McMurry et al., 1980; Van Bambeke et al., 2003).

A synergistic effect of CPZ with gentamicin or neomycin was not observed, which suggests the occurrence of other resistance mechanisms or of a CPZ-insensitive efflux pump that can be blocked by EEMC (Table 1).

Table 2 shows the results for the combinations of antibiotics and EEMC, at or below their MIC. EEMC itself had no inhibitory activity against the *E. coli* strain tested. Synergism was observed with the combination of EEMC and kanamycin. The combinations of EEMC and amikacin, gentamicin, and neomycin gave varied responses. Amikacin and neomycin demonstrated an additive effect, but the effect was indifferent with gentamicin.

The results obtained indicate that *Momordica charantia* could serve as a source of plant-derived natural products with antibiotic resistance-modifying activity to be used against multidrug-resistant bacteria, as with chlorpromazine.

**Table 1.** MIC* values (μg/mL) of aminoglycosides in the absence and presence of EEMC† and CPZ‡ in *Escherichia coli* 27.

| | EC 27 | | |
|---|---|---|---|
| | | Combined MIC | |
| Antibiotic | MIC alone | EEMC (128 μg/mL) | CPZ (16 μg/mL) |
| Amikacin | 64 | ≤ 1 | 16 |
| Gentamicin | 4 | ≤ 1 | 4 |
| Kanamycin | 256 | 8 | 16 |
| Neomycin | 8 | ≤ 1 | 8 |
| Chlorpromazine | 64 | — | — |

*MIC, minimal inhibitory concentration; †EEMC, ethanolic extract of *Momordica charantia*; ‡CPZ, chlorpromazine.

**Table 2.** MIC* of antibiotics and the effect of combinations with EEMC† against *Escherichia coli* 27.

| Antibiotic + EEMC | MIC (μg/mL) | FIC‡ index (type of interaction) |
|---|---|---|
| EEMC | 512 | |
| Amikacin | 64 | |
| Kanamycin | 128 | |
| Gentamicin | 4 | |
| Neomycin | 32 | |
| EEMC/amikacin | 32/32 | 0.5625 (additive) |
| EEMC/kanamycin | 64/32 | 0.375 (synergistic) |
| EEMC/gentamicin | 32/4 | 1.0625 (indifferent) |
| EEMC/neomycin | 128/16 | 0.75 (additive) |

*MIC, minimal inhibitory concentration; †EEMC, ethanolic extract of *Momordica charantia*; ‡FIC, fractional inhibitory concentration.

***Declaration of interest*:** The authors report no conflicts of interest.

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