International Food Research Journal 22(5): 1777-1782 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

# Carotenoids and flavonoids can impair the effectiveness of some antimicrobial drugs against clinical isolates of *Escherichia coli* and *Staphylococcus aureus*

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**Article history**

*Received: 25 February 2015*
*Received in revised form: 31 March 2015*
*Accepted: 23 April 2015*

**Keywords**

*Phytonutrients*
*Interaction*
*Antimicrobials*

**Abstract**

Patients are taking more control over their health, and attracted by contributions to human nutrition, the use of phytonutrients raised. Carotenoids and flavonoids are among the most studied and consumed chemical entities in this sense. However, patients are often unaware of the potential risks associated to drug-herbal interactions. In this study, four drugs were tested combined to lycopene, β-carotene, resveratrol and rutine in an *in vitro* model against clinical isolates of *E. coli* and *S. aureus*. Statistically significant antagonism was detected within most of the tested interactions for at least one isolate. Few events of synergism were detected. For *E. coli*, the antimicrobial activity of Cephalexin and Amoxicillin was significantly reduced. For *S. aureus*, strong reduction of the antimicrobial activity was seen for all drugs. Our data suggest that there might be risks of impairment of the antimicrobial activity of these drugs if co-administered with the tested phytonutrients. Further complementary *in vivo* studies are needed for full assessment of the biological effects of these interactions.



## Introduction

In the latest decades, more individuals are taking control over their health for treating and preventing diseases, especially if they are chronic or if they do not necessarily demand treatments in hospital settings. One of the most adopted health promotion measures for these purposes has been the rise on the use of phytonutrients, which are specific components obtained from plant food that can be employed for several health benefits through oral or topical use. Varied pharmacological activities of interest have been described for many phytonutrients, and several pharmaceutical formulations have been developed with these compounds in order to explore them clinically, as herbal remedies or phytomedications (Mouly *et al*., 2015; Kreft *et al*., 2015).

Many phytonutrients-based products are sold without need of medical prescription worldwide. The advances of media technologies favored a wide dissemination of the outstanding (and often unique) physiologic and biochemical contributions of phytonutrients to human nutrition, what helps to explain the rise in consumption. Carotenoids and flavonoids are among the most studied and consumed chemical entities nowadays as both nutrients and phytomedication (Pápai *et al*., 2010; Rodrigues *et al*., 2013).

Carotenoids are tetraterpenic pigments lacking symmetry, produced by plants with a vital role in photosynthesis, protection from photo-oxidation and in plant–animal communication. Some bacteria and fungi are also producers of these molecules. Lycopene and β-carotene are among the most studied substances of the group. Carotenoids are responsible for the color of many fruits and flowers, and currently, their most important biological activities are the antioxidant potential and being precursors of vitamin A and its derivatives retinoic acid and retinal. Recent studies also suggests that the antitumor and antioxidant activities of these carotenois consists in quenching singlet oxygen and scavenging reactive oxygen species (ROS), minimizing cell damage and preventing the development of different types of cancer (Boullata *et al*., 2012; Berginc *et al*., 2015).

Flavonoids are present in many plant foods and have assumed great relevance in the latest years due to antioxidant, anti-inflammatory, antimicrobial, anti-platelet, cardioprotective and antitumor properties. Some chemical subgroups are more commonly detected in plant food, like flavanones, anthocyanidins, isoflavones, flavones and flavonols. Different works have provided evidence that the biological properties of flavonoids and their ingestion

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can have a protective effect against the progression of several diseases like hypertension, diabetes and malignant neoplasms (Won *et al.*, 2012; Conner *et al.*, 2013).

Despite the advantages of the ingestion of phytonutrients, a concern arises when patients are using medications combined to natural products. Although considerable quantities of flavonoids and carotenoids are consumed daily in human vegetable diet and adverse effects have not been described so far, many interactions of drugs and molecules of natural sources remain speculative and poorly described; therefore, predicting positive or negative effects of the combined use of drugs and molecules of natural sources becomes a difficult endeavor. Antimicrobial drugs represent a particular problem in this subject matter: many bacterial infections are of difficult treatment because of multidrug resistance, and negative effects due to interactions can lead to complex clinical pictures of difficult treatment because of bacterial growth with strong drug resistance (Conner *et al*., 2013; Dias-Souza *et al.*, 2013).

Considering the limited knowledge on interactions of phytonutrients with antimicrobial drugs, we investigated the effects of the combined use of lycopene, resveratrol and rutine with certain antimicrobials in an *in vitro* model against clinical isolates of *Escherichia coli* and *Staphylococcus aureus*. Here we show by the first time that despite these phytonutrients had no antimicrobial activity against the clinical isolates, the tested interactions resulted in strong reduction of the pharmacological activity of the antimicrobial drugs, with rare occurrences of synergism in clinical samples. Our data open doors for clinical counseling of patients and professionals regarding such interactions.

## Materials and Methods

*Microorganisms*

Ethical approval was obtained prior to the commencement of the study (University Vale do Rio Doce Ethics Committee, PQ 024/10-10). Isolates of *E. coli* consisted of uropathogenic samples and isolates of *S. aureus* were from catheter tips, both obtained of adult patients. Samples (10 of each species) were kindly provided by Dr Pedro Marçal from the clinical isolates collection from the Microbiology Research Laboratory, University Vale do Rio Doce. All isolates were cultured overnight in BHI broth (Difco) at 35±2°C for activation, and tested with Gram-positive and Gram-negative bacteria identification cards for VITEK 2 system (bioMérieux) for identity confirmation. Each card was inoculated with a bacterial suspension prepared in saline solution from manufacturer's kit and analyzed according to the manufacturer's instructions.

*Interference test*

The possible interference of phytonutrients on the antimicrobial drugs was assessed as described by Dias-Souza *et al.* (2013), with some modifications. Lycopene, β-carotene, resveratrol and rutine were purchased from Fagron (Brazil) in analytical grade, and solutions were prepared in hot DMSO (analytical grade) based in common concentrations of these nutrients consumed in Brazil as manufactured formulations: 15 mg/mL for lycopene (Moritz *et al*., 2006), 30 mg/mL for β-carotene (Fisberg *et al*., 2008) and 60 mg/mL for flavonoids (Arabbi *et al*., 2004).

The interference assay was performed in duplicate. Agar plates were prepared with Mueller-Hinton agar (Difco). Antimicrobial disks (gentamycin 10 µg, amoxicillin 10 µg, cephalexin 30 µg and ciprofloxacin 5 µg against E. coli - and nitrofurantoin 300 µg, penicillin 10 U, erythromycin 15 µg and oxacillin 5 µg against S. aureus, all from Sensifar) were distributed as for performing an antimicrobial susceptibility assay as recommended by the CLSI (2009). Following, briefly, 10 µL of each phytonutrient solution was then dispensed in each disk. Plates were incubated overnight at 35±2 °C, and the inhibition zone mean diameter was compared with control plates (disks free of phytonutrients). Synergism was considered if the inhibition zone mean diameter was at least 2 mm larger than the control, and antagonism was considered if the inhibition zone mean diameter was at least 2 mm shorter than the control. If the inhibition zone mean diameters were larger or shorter than the control but no significant difference was seen, data was described as tendency of synergism or antagonism.

*Assessment of the antimicrobial activity of the phytonutrients*

This assay was performed in duplicate. Overnight strains cultured in BHI agar (Difco) were transferred to Mueller Hinton Broth (Oxoid) and the turbidity was adjusted as a 0.5 McFarland standard. Strains were then dispensed in 96-wells polystyrene plates, using 100 µL in three wells for each bacterial sample. For each repetition of three wells, 100 µL of solutions of each phytonutrient in the concentrations of 100 and 500 µg/mL were dispensed. The plates were then incubated overnight at 35±2°C. Bacterial growth was analyzed through viability staining using 50 µL of a 0.1% resazurine solution (Sigma). Pink color

Table 1. Interference of flavonoids and carotenoids over antimicrobial drugs against *E. coli* isolates

| Strain | GEN | GEN+ LYC | GEN+β | AMOX | AMOX+LYC | AMOX+β | CPH | CPH+LYC | CPH+β | CIP | CIP+LYC | CIP+β |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Escherichia coli 1* | 16 | 10 † | 10† | 27 | 24.5 ± 0.7 ‡ | 31.5 ± 0.12 Δ | 19.5 ± 0.7 | 19 | 18 | 26.5 ± 0.12 | 24.5 ± 0.7 | 27.5 ± 0.7 |
| *Escherichia coli 2* | 14.5 ± 0.7 | 15 | 15 | 11.5 ± 0.7 | 0 ‡ | 0 ‡ | 20.5 ± 0.7 | 0 ‡ | 0 ‡ | 29 ± 0.41 | 30 | 32 |
| *Escherichia coli 3* | 14 | 16.5 Δ | 15 | 15 | 15.5 ± 0.7 | 15.5 ± 0.7 | 15.5 ± 0.7 | 0 ‡ | 15.5 ± 0.7 | 24.5 ± 0.7 | 19.5 ± 0.7 ‡ | 23.5 ± 0.7 |
| *Escherichia coli 4* | 13 | 12 | 12 | 13.5 ± 0.7 | 0 ‡ | 0 ‡ | 18 | 0 ‡ | 0 ‡ | 23 | 29.5 ± 0.7 Δ | 29 |
| *Escherichia coli 5* | 13.5 ± 0.7 | 15± 0.7 Δ | 15 | 14 | 0 ‡ | 0 ‡ | 18.5 ± 0.7 | 0 ‡ | 0 ‡ | 29 ± 0.41 | 29 | 27.5 ± 0.7 † |
| *Escherichia coli 6* | 11 | 14 Δ | 11 | 13 | 0 ‡ | 0 ‡ | 22.5 ± 0.7 | 0 ‡ | 0 ‡ | 32.5 ± 0.53 | 35 ± 0.7 * | 49 ± 0.7 * |
| *Escherichia coli 7* | 10 | 12 Δ | 12 Δ | 23.5 ± 0.5 | 20 ‡ | 25.5 ± 0.7 Δ | 16 | 14.5 ± 0.7‡ | 13.5 ± 0.7 ‡ | 15.5 ± 0.7 | 24.5 ± 0.12 Δ | 28 Δ |
| *Escherichia coli 8* | 15 | 17± 0.7 Δ | 15.5 ± 0.7 | 16 | 15.5 ± 0.7 | 15.5 ± 0.7 | 15.5 ± 0.7 | 17 * | 18.5 ± 0.7 * | 28 | 25.5 ± 0.53 † | 24 ± 0.41 |
| *Escherichia coli 9* | 10 | 15.5 Δ | 16.5 ± 0.7 Δ | 17 | 17 | 15.5 ± 0.7† | 16.5 ± 0.7 | 16.5 ± 0.7 | 16 ± 0.7 | 25 ± 0.82 | 28 Δ | 31 ± 0.41 |
| *Escherichia coli 10* | 13 | 15 Δ | 15 Δ | 14 | 15.5 ± 0.7* | 17.5 ± 0.7 Δ | 14 | 15.5 ± 0.7 | 12 ± 0.7 ‡ | 21 | 21.5 ± 0.7 | 22 ± 0.41 |
| | GEN | GEN+RES | GEN+RUT | AMOX | AMOX+RES | AMOX+RUT | CPH | CPH+RES | CPH+RUT | CIP | CIP+RES | CIP+RUT |
| *Escherichia coli 1* | 16 | 10 ‡ | 7.5 ± 0.7‡ | 27 | 25.5 ± 0.7‡ | 25 ‡ | 19.5 ± 0.7 | 19 | 18 | 26.5 ± 0.12 | 21±0.41‡ | 23.5 ± 0.7 |
| *Escherichia coli 2* | 14.5 ± 0.7 | 17 | 10.5 ± 0.7‡ | 11.5 ± 0.7 | 0 ‡ | 0 ‡ | 20.5 ± 0.7 | 0 ‡ | 0 ‡ | 29 ± 0.41 | 34* | 30 |
| *Escherichia coli 3* | 14 | 17 | 11‡ | 15 | 15 | 16 | 15.5 ± 0.7 | 15 | 17* | 24.5 ± 0.7 | 26 Δ | 27 Δ |
| *Escherichia coli 4* | 13 | 10.5±0.7‡ | 10‡ | 13.5 ± 0.7 | 0 ‡ | 0 ‡ | 18 | 0 ‡ | 0 ‡ | 23 | 30* | 26 ‡ |
| *Escherichia coli 5* | 13.5 ± 0.7 | 9.5 ± 0.7 | 10.5 ± 0.7‡ | 14 | 0 ‡ | 0 ‡ | 18.5 ± 0.7 | 0 ‡ | 0 ‡ | 29 ± 0.41 | 29 | 28 |
| *Escherichia coli 6* | 11 | 13 | 9.5 ± 0.7‡ | 13 | 0 ‡ | 0 ‡ | 22.5 ± 0.7 | 0 ‡ | 0 ‡ | 32.5 ± 0.53 | 30 ‡ | 36* |
| *Escherichia coli 7* | 10 | 9.5 ± 0.7 | 8 † | 23.5 ± 0.5 | 21.5 ± 0.7 | 26 * | 16 | 13.5±0.7 ‡ | 11.5 ± 0.7 ‡ | 15.5 ± 0.7 | 24±0.41* | 23±0.82* |
| *Escherichia coli 8* | 15 | 15.5 ± 0.7 | 15.5 ± 0.7 | 16 | 16.5 ± 0.7 | 16.5 ± 0.7 | 15.5 ± 0.7 | 13±0.41 ‡ | 15 | 28 | 21 ‡ | 19.5±0.12‡ |
| *Escherichia coli 9* | 10 | 15.5±0.7* | 15 * | 17 | 16 | 17 | 16.5 ± 0.7 | 15 | 15.5 ± 0.7 | 25 ± 0.82 | 26 | 25 |
| *Escherichia coli 10* | 13 | 12 | 10 ‡ | 14 | 16.5 ± 0.7* | 17 * | 14 † | 17 * | 15.5 ± 0.7 ‡ | 21 | 21.5 ± 0.7 | 23±0.41 Δ |

Data is presented ±Standard deviation. GEN: Gentamycin; AMOX: Amoxicillin; CPH: Cephalexin; CIP: Ciprofloxacin. +LYC: Addition of Lycopene; +β: Addition of β-carotene; +RES: Addition of Resveratrol; +RUT: Addition of Rutine; *: Synergism, statistically significant; ‡: Antagonism, statistically significant; Δ: Synergism tendency (no statistical significance); †: Antagonism tendency (no statistical significance); Absence of signals indicate no difference when compared to the control group

indicated bacterial growth, and blue color indicated effective antimicrobial activity. Wells with cultures free of phytonutrients and fresh media were used as positive and negative controls respectively.

*Statistical analysis*

All analyses were carried out in Minitab 17 statistical package for Windows. Homocedasticity and normality were assessed through Bartlett's test and through Shapiro-Wilk test respectively. Mean diameters of the inhibition zones with and without addition of phytonutrients were analyzed using ANOVA followed by post-hoc Tukey test. The significance level was set at $p \leq 0.05$.

## Results

For both species, statistically significant antagonism was detected within most of the tested interactions for at least one isolate in each association. On the other hand, events of statistically significant synergism of phytonutrients and drugs were rare, and some tendencies (i.e., differences statistically not significant when compared to the controls) of synergism and antagonism were registered. For *E. coli* strains, the antimicrobial activity of Cephalexin and Amoxicilin was significantly reduced in most of the tested strains when any of the tested interfering phytonutrients was combined to them. However, few antagonistic interference events were detected for Gentamycin and Ciprofloxacin, and most of the results of synergism and antagonism tendencies were observed for these drugs (Table 1). For *S. aureus,* strong reduction of the antimicrobial activity was seen for all tested drugs, and all drugs had at least one isolate without formation of inhibition zones. No synergism was detected for any of the tested combinations against the strains (Table 2).

Most of the published data on the pharmacological activity of the phytonutrients we tested are related to antioxidant and anti-proliferative activities, and to protective effects on varied biological tissues (Arabbi *et al.*, 2004; Fisberg *et al.*, 2008). Therefore, considering our interference data, we sought to investigate if the phytonutrients used in this study would have any antimicrobial activity against the clinical isolates. As the results of this experiment would give us some directions on the understanding of the interference test, we cultured each isolate with solutions of 100 and 500 µg/mL of each phytonutrient, and assessed bacterial growth through resazurine staining (data not shown). Interestingly, none of the phytonutrients showed antimicrobial activity against any strain in the tested concentrations.

## Discussion

The majority of the tested drug-phytonutrient interactions were characterized as antagonism (Figure 1), and significant differences were found when compared with the control. Our data suggest that there may be risks of impairment of the antimicrobial activity of such drugs if these phytonutrients are used in combination to them. This becomes even more important for both nutritional and pharmaceutical counseling on drug-nutrient interaction risks. The research on interactions of natural products and synthetic antimicrobials has been carried out mostly

Table 2. Interference of flavonoids and carotenoids over antimicrobial drugs against *S. aureus* isolates

| Strain | NIT | NIT+LYC | NIT+β | PEN | PEN+LYC | PEN+β | ERI | ERI+LYC | ERI+β | OXA | OXA+LYC | OXA+β |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Staphylococcus aureus 1* | 17±0.41 | 6‡ | 8‡ | 12±0.4 | 0‡ | 0‡ | 19±.0 | 0‡ | 0‡ | 14.5±0.7 | 0‡ | 0‡ |
| *Staphylococcus aureus 2* | 14.5±0.7 | 0‡ | 0‡ | 13±0.42 | 0‡ | 0‡ | 13.5±0.7 | 0‡ | 0‡ | 15 | 0‡ | 0‡ |
| *Staphylococcus aureus 3* | 14.5±0.7 | 0‡ | 0‡ | 16.5±0.7 | 12‡ | 0‡ | 12 | 9.5±0.7‡ | 7.5±0.7† | 10.5±0.7 | 0‡ | 0‡ |
| *Staphylococcus aureus 4* | 14.5±0.7 | 0‡ | 11±0.4‡ | 17±0.4 | 10.5±0.7‡ | 0‡ | 15 | 0‡ | 0‡ | 15.5±0.7 | 0‡ | 0‡ |
| *Staphylococcus aureus 5* | 12.5±0.7 | 0‡ | 0‡ | 17.5±0.7 | 0‡ | 11.5±0.7‡ | 14.5±0.7 | 0‡ | 0‡ | 11.5±0.7 | 0‡ | 0‡ |
| *Staphylococcus aureus 6* | 15 | 14.5±0.7 | 10.5±0.7‡ | 19.5±0.7 | 9‡ | 0‡ | 15 | 0‡ | 0‡ | 13.5±0.7 | 0‡ | 0‡ |
| *Staphylococcus aureus 7* | 15.5±0.7 | 0‡ | 0‡ | 18.5±0.7 | 10‡ | 0‡ | 14 | 0‡ | 0‡ | 12.5±0.7 | 0‡ | 0‡ |
| *Staphylococcus aureus 8* | 12.5±0.7 | 0‡ | 0‡ | 12.5±0.7 | 0‡ | 0‡ | 13.5±0.7 | 0‡ | 0‡ | 13.5±0.7 | 0‡ | 0‡ |
| *Staphylococcus aureus 9* | 12.5±0.7 | 14.5±0.7Δ | 0‡ | 20 | 9‡ | 8.5±0.7‡ | 14.5±0.7 | 0‡ | 0‡ | 14±0.4 | 0‡ | 0‡ |
| *Staphylococcus aureus 10* | 14.5±0.7 | 14.5±0.7 | 0‡ | 14.6±0.7 | 12.5±0.7† | 8.5±0.7‡ | 20 | 0‡ | 0‡ | 12.5±0.7 | 0‡ | 0‡ |
| | NIT | NIT+RES | NIT+RUT | PEN | PEN+RES | PEN+RUT | ERI | ERI+RES | ERI+RUT | OXA | OXA+RES | OXA+RUT |
| *Staphylococcus aureus 1* | 17±0.41 | 14‡ | 6.5±0.7‡ | 12±0.4 | 0‡ | 0‡ | 19±.0 | 0‡ | 0‡ | 14.5±0.7 | 0‡ | 0‡ |
| *Staphylococcus aureus 2* | 14.5±0.7 | 0‡ | 0‡ | 13±0.42 | 8.5±0.7‡ | 10.5±0.7‡ | 13.5±0.7 | 0‡ | 0‡ | 15 | 0‡ | 0‡ |
| *Staphylococcus aureus 3* | 14.5±0.7 | 0‡ | 0‡ | 16.5±0.7 | 12.5±0.7‡ | 11‡ | 12 | 0‡ | 0‡ | 10.5±0.7 | 0‡ | 0‡ |
| *Staphylococcus aureus 4* | 14.5±0.7 | 11.5±0.7‡ | 12.5±0.7 | 17±0.4 | 10.5±0.7‡ | 0‡ | 15 | 0‡ | 0‡ | 15.5±0.7 | 0‡ | 0‡ |
| *Staphylococcus aureus 5* | 12.5±0.7 | 0‡ | 15.5±0.7 | 17.5±0.7 | 0‡ | 0‡ | 14.5±0.7 | 0‡ | 0‡ | 11.5±0.7 | 0‡ | 0‡ |
| *Staphylococcus aureus 6* | 15 | 0‡ | 0‡ | 19.5±0.7 | 0‡ | 0‡ | 15 | 0‡ | 0‡ | 13.5±0.7 | 0‡ | 0‡ |
| *Staphylococcus aureus 7* | 15.5±0.7 | 10‡ | 0‡ | 18.5±0.7 | 8.5±0.7‡ | 0‡ | 14 | 0‡ | 0‡ | 12.5±0.7 | 0‡ | 0‡ |
| *Staphylococcus aureus 8* | 12.5±0.7 | 7‡ | 0‡ | 12.5±0.7 | 0‡ | 0‡ | 13.5±0.7 | 0‡ | 0‡ | 13.5±0.7 | 0‡ | 0‡ |
| *Staphylococcus aureus 9* | 12.5±0.7 | 0‡ | 0‡ | 20 | 10‡ | 10.5±0.7‡ | 14.5±0.7 | 0‡ | 0‡ | 140.4 | 0‡ | 0‡ |
| *Staphylococcus aureus 10* | 14.5±0.7 | 0‡ | 0‡ | 14.5±0.7 | 8‡ | 0‡ | 20 | 0‡ | 0‡ | 12.5±0.7 | 0‡ | 0‡ |

Data is presented as Mean ±Standard deviation; NIT: nitrofurantoin; PEN: penicillin; ERI: erythromycin; OXA: oxacillin; +LYC: Addition of Lycopene; +β: Addition of β-carotene; +RES: Addition of Resveratrol; +RUT: Addition of Rutine; *:Synergism, statistically significant; ‡: Antagonism, statistically significant; Δ: Synergism tendency (no statistical significance); †: Antagonism tendency (no statistical significance); Absence of signals indicate no statistical difference when compared to antimicrobial discs of the control group

with plant extracts, and few works have investigated the possible effects of isolated molecules in this context. In this work, we have shown by the first time that lycopene, β-carotene, resveratrol and rutine, which are phytonutrients present in food widely consumed in Brazil like tomatoes, carrots, grapes and apple (respectively), and are also used as phytomedications for varied therapeutic purposes, can interfere in the antimicrobial activity of some drugs of clinical interest.

The rise in the concomitant use of medications and herbal products or nutritional supplements can lead to considerably increased risks of adverse effects due to negative interactions. Currently, our understanding on the potential of interactions between molecules from natural sources and synthetic drugs is very limited, primarily due to difficulties in elucidating the possible mechanisms involved, what also impairs predicting such events. Patients who choose to use herbal products and are following a drug therapy scheme are often not aware of possible adverse reactions. The large use of certain herbal products has pointed out that they are not free of risks as commonly stated among many patients worldwide (Mouly *et al.*, 2015).

Sato *et al*. (2006) have shown synergism of isoflavones isolated from *Erythrina poeppigiana* combined to vancomycin against methicillin-resistant *S. aureus* (MRSA). Of the isolated isoflavones, isolupalbigenin and erythrinin B exhibited the highest anti-MRSA activity. Moreover, Mun *et al.* (2014) investigated the antimicrobial activity of carvone enantiomers R-car and S-car combined to Gentamycin against MRSA. Carvone is a naturally occurring monoterpene widely distributed in several plants. The combined molecules R-car/S-car, R-car/Gentamycin and S-car/Gentamycin exhibited significant synergistic activity against MRSA. Minimal inhibitory concentration values for R- and S-car against six different strains of *S. aureus* ranged between 0.5 and 1 mg/ml.

Gould *et al.* (2009) reported the antimicrobial activities of pomegranate rind extract free and combined to cupric sulphate against methicillin-sensitive and resistant *S. aureus*, and Panton-Valentine Leukocidin positive community acquired MSSA. Pomegranate rind extract showed limited efficacy against MRSA and MSSA strains when used alone. Exposure to copper (II) ions alone for 2 hours resulted in moderate antimicrobial activity against the isolates, which was enhanced by the addition of pomegranate rind extract. Reduction in growth was observed for 80% of the isolates.

Jayaraman *et al.* (2010) assessed the interference in vitro of the phytonutrients protocatechuic acid, gallic acid, ellagic acid, rutin, berberine and myricetin in the antimicrobial activity of ciprofloxacin, ceftazidime, tetracycline, trimethoprim, sulfamethoxazole, polymyxin B and piperacillin, against five *P. aeruginosa* isolates. The combinations of sulfamethoxazole and protocatechuic acid, sulfamethoxazole and ellagic acid, sulfamethoxazole and gallic acid, and tetracycline and gallic acid demonstrated inhibitory activity against the clinical

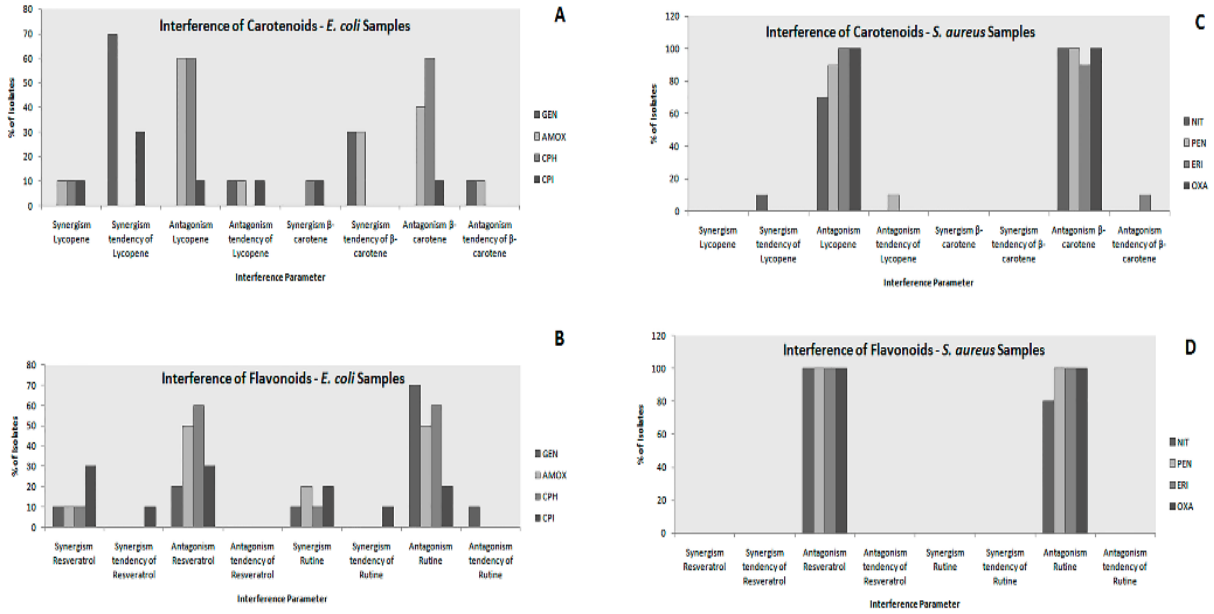


Figure 1. Summarization of interference parameters results. Data is presented as percentage of isolates included in each interference parameter for carotenoids (A – *E. coli* samples; C – *S. aureus* samples) and flavonoids (B – *E. coli* samples; D – *S. aureus* samples). GEN: Gentamycin; AMOX: Amoxicillin; CPH: Cephalexin; CIP: Ciprofloxacin; NIT: nitrofurantoin; PEN: penicillin; ERI: erythromycin; OXA: oxacillin

isolates.

The *in vitro* antimicrobial activity of pannarin, a phenolic molecule (from the depsidone family) isolated from lichens, was evaluated by Celenza *et al.* (2012) in combination with some antimicrobials against MRSA. The authors observed moderate synergistic action when pannarin was combined to Gentamycin, whilst antagonism was observed when combined to levofloxacin.

Interactions between the methanolic extract of *Acacia mearnsii* and eight antimicrobials were investigated by Olajuyigbe and Afolayan (2012). The synergistic interaction was most expressed by combining the extract with tetracycline, metronidazole, amoxicillin, ciprofloxacin, chloramphenicol and nalidixic acid against *E. coli* (ATCC 6538), and erythromycin, metronidazole, amoxicillin, chloramphenicol and kanamycin against *S. aureus* (ATCC 25922).

Previous work of our group have shown that the hydroethanolic extract of cashew *(Anacardium occidentale)* stem bark, which is rich in flavonoids, saponins, anacardic acid and tannins and has also antibiofilm activity, can also reduce the antimicrobial activity of varied drugs against clinical isolates of *S. aureus* strains (Dias-Souza *et al*., 2013). Furthermore, Hussin and El-Sayed (2011) explored the interactions of dichloromethane/methanol extracts of *Punica granatum, Thymus vulgaris, Commiphora molmol* and *Achillea fragrantissima* combined to tetracycline, against *S. aureus* (ATCC 25923), *Bacillus megaterium* (ATCC 14591), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) and *Klebsiella pneumonia* (ATCC 700603) strains. All extracts had significant antimicrobial activity against all tested bacteria and increased significantly the post antibiotic effect.

This study, nevertheless, has some limitations. The concentrations of the tested phytonutrients are related to their consumption in Brazil; therefore, different concentrations should be tested for more generalisable results. Also, our data was related to four drugs which are widely used in hospital settings, but other drugs can be considered. Although we could provide statistical support for our observations, the method we explored in this research is qualitative; quantitative approaches such as the checkerboard method are being explored by our group to conduct further researches based on the results we described in this paper.

## Conclusions

We have shown for the first time that lycopene, β-carotene, resveratrol and rutine can interfere in the antimicrobial activity of determined drugs against *E. coli* and *S. aureus* clinical strains. Our initial studies indicate that there can be risks of impairment of clinical treatments with the tested antimicrobial drugs if they are co-administered with the tested phytonutrients, what can contribute

to bacterial resistance. Further complementary in vivo studies are needed for a complete assessment of the biological effects of these interactions, and the molecular mechanisms that are responsible for the observed effects remains to be determined. Even so, these technical limitations have not impaired our measuring of the responsiveness of the methods used in this study, neither their potential of predicting the pharmacological results of interactions expressed in synergism and antagonism.

**Acknowledgements**

We are thankful to Dr Pedro Marçal (University Vale do Rio Doce, MG), for kindly providing access to the clinical strains and support to part of our analyses. MVDS is supported by grants from Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG).

**References**

Arabbi, P.R., Genovese, M.I. and Lajolo, F.M. 2004. Flavonoids in Vegetable Foods Commonly Consumed in Brazil and Estimated Ingestion by the Brazilian Population. Journal of Agricultural Food Chemistry (52): 1124-1131.

Berginc, K. 2015. Pharmacokinetic interactions between drugs and dietary supplements: herbal supplements. In: Berginc, K. and Kreft, S. (Eds). Woodhead Publishing Series in Food Science, Technology and Nutrition, Dietary Supplements, p. 47-68. Amsterdan: Elsevier.

Boullata, J.I. and Hudson, L.M. 2012. Drug–Nutrient Interactions: A Broad View with Implications for Practice. Journal of the Academy of Nutrition and Dietetics 112 (4): 506-517.

Celenza, G., Segatore, B., Setacci, D., Bellio, P., Brisdelli, F., Piovano, M., Garbarino, J.A., Nicoletti, M., Perilli, M. and Amicosante, G. 2012. *In vitro* antimicrobial activity of pannarin alone and in combination with antibiotics against methicillin-resistant *Staphylococcus aureus* clinical isolates. Phytomedicine 19(7): 596-602.

Clinical and Laboratory Standards Institute. 2009. Performance standard for antimicrobial disk susceptibility tests. M2-A10. CLSI, Wayne, PA.

Conner, K.G. 2013. Drug–Nutrient Interactions. In: Caballero, B. (Ed). Encyclopedia of Human Nutrition (Third Edition), p. 1904-1912. Waltham: Academic Press.

Dias-Souza, M.V., Caldoncelli, J.L. and Monteiro, A.S. 2013. *Annacardium occidentale* Stem Bark Extract can Decrease the Efficacy of Antimicrobial Drugs. Journal of Medical and Biological Sciences (12): 161-165.

Fisberg, R.M., Colucci, A.C.A., Morimoto, J.M. and Marchioni, D.M.L. 2008. Questionário de freqüência alimentar para adultos com base em estudo populacional. Revista de Saúde Pública 42(3): 550-554.

Gould, S.W., Fielder, M.D., Kelly, A.F. and Naughton, D.P. 2009. Anti-microbial activities of pomegranate rind extracts: enhancement by cupric sulphate against clinical isolates of *S. aureus,* MRSA and PVL positive CA-MSSA. BMC Complementary and Alternative Medicine 9(23): doi:10.1186/1472-6882-9-23.

Hussin, W.A. and El-Sayed, W.M. 2011. Synergic Interactions Between Selected Botanical Extracts and Tetracycline Against Gram Positive and Gram Negative Bacteria. Journal of Biological Science (11): 433-441.

Jayaraman, P., Sakharkar, M.K., Lim, C.S., Tang, T.H. and Sakharkar, K.R. 2010. Activity and interactions of antibiotic and phytochemical combinations against *Pseudomonas aeruginosa in vitro*. International Journal of Biological Sciences 6(6): 556-568.

Kreft, S. 2015. Pharmacodynamic interactions between drugs and dietary supplements. In: Berginc, K. and Kreft, S. (Eds). Woodhead Publishing Series in Food Science, Technology and Nutrition, Dietary Supplements, p. 127-135. Amsterdan: Elsevier.

Moritz, B. and Tramonte, V.L.C. 2006. Biodisponibilidade do licopeno. Revista de Nutrição 19(2): 265-273.

Mouly, S., Morgand, M., Lopes, A., Lloret-Linares, C. and Bergmann, J.F. 2015. Interactions médicaments–aliments en médecine interne: quels messages pour le clinicien? La Rev Méd Internet Available online Jan 27, 2015 (in press).

Mun, S.H., Kang, O.H., Joung, D.K., Kim, S.B., Choi, J.G., Shin, D.W. and Kwon, D.Y. 2014. *In vitro* anti-MRSA activity of carvone with Gentamycin. Experimental and Therapeutic Medicine 7(4): 891-896.

Olajuyigbe, O.O. and Afolayan, A.J. 2012. Pharmacological Assessment of the Medicinal Potential of Acacia mearnsii De Wild.: Antimicrobial and Toxicity Activities. International Journal of Molecular Science 13(4): 4255–4267.

Pápai, K., Budai, M., Ludányi, K., Antal, I. and Klebovich, I. 2010. *In vitro* food–drug interaction study: Which milk component has a decreasing effect on the bioavailability of ciprofloxacin? Journal of Pharmaceutical and Biomedical Analysis 52(1): 37-42.

Rodrigues, M., Alves, G. and Falcão, A. 2013. Investigating herb–drug interactions: The effect of *Citrus aurantium* fruit extract on the pharmacokinetics of amiodarone in rats. Food Chemistry and Toxicology (60): 153-159.

Sato, M., Tanaka, H., Tani, N., Nagayama, M. and Yamaguchi, R. 2006. Different antibacterial actions of isoflavones isolated from *Erythrina poeppigiana* against methicillin-resistant Staphylococcus aureus. Letters in Applied Microbiology 43: 243–248.

Won, C.S., Oberlies, N.H. and Paine, M.F. 2012. Mechanisms underlying food–drug interactions: Inhibition of intestinal metabolism and transport. Pharmacology and Therapeutics 136(2): 186-201.