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## INTRODUCTION

Bacterial resistance to antibiotics has increased alarmingly over the last few decades due to the overuse of these drugs. On the contrary, the benefits of natural products of vegetable origin have been known and used for multiple applications, including empirical treatments of infections, for centuries without generating bacterial resistance.

**AIM:** The aim of this work was to study the resistome of two multidrug resistant *Escherichia coli* strains isolated from the natural intestinal microbiota of healthy animals, and the effect of a red grape polyphenol extract on the growth of these multidrug resistant *E. coli* strains.

## MATERIALS AND METHODS

**Whole genome analysis** of two *E. coli* strains C6898 and C6840 was performed by library construction and sequencing paired-end 100 bp reads in an Illumina HiSeq 1500 system. Chromosome and plasmid reconstruction was performed with the PLACNETw web-based tool (1).

**Antimicrobial activity:** *E. coli* strains were tested by the broth microdilution method (2), and minimal inhibitory concentration (MIC) values were determined in BHI broth. The effect of a polyphenol extract from skins of Graciano red grapes (GG) (Table 1) on the growth of C6898 and C6840 was studied. The effect of two pure polyphenols: malvidin and epicatechin was also studied.

**Synergistic study:** Synergistic effects of Graciano grape extract (GG) with the antibiotic of reference ampicillin (AMP) against the multiresistant strain C6840, was studied by the checkerboard method and fractional inhibitory concentrations (FIC) were determined (3).

Table 1. Phenolic composition of the Graciano Grape extract (GG).

Polyphenol	Graciano grape extract (GG) content (mg/g of extract)
<b>TOTAL Flavonols</b>	162,9 ± 3,8
Myricetin	97,5 ± 2,0
Quercetin	36,4 ± 0,6
Kaempferol	29,0 ± 1,3
<b>TOTAL Anthocyanins</b>	402,5 ± 12,6
Delphinidin	64,8 ± 1,2
Cyanidin	32,1 ± 1,7
Petunidin	63,7 ± 1,7
Peonidin	97,6 ± 3,6
Malvidin	144,3 ± 4,4



Figure 1. Genome reconstruction using the PLACNETw web-based tool for the strain C6898 (a) and C6840 (b). ● Reference node; ● contig node

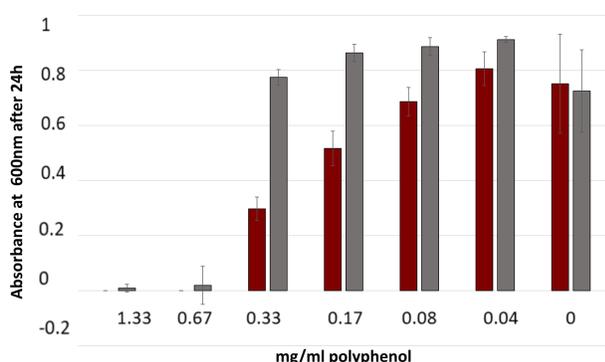


Figure 2. Effect of polyphenol concentration on the growth of *E. coli* C6840 with: ■ malvidin and ■ epicatechin.

## RESULTS AND DISCUSSION

**Genomic study:** The genomes of both strains were reconstructed (Figure 1). Strain C6840 showed 59 genes related to antibiotic resistances, two of which were located in its plasmid. Strain C6898 showed 48 genes related to antibiotic resistances in its chromosome, and 6 more resistance genes in each of its two plasmids. Both strains showed very high resistance to the antibiotic of reference AMP (MIC values > 3 mg/mL).

**Antimicrobial activity:** The inhibitory effect of pure malvidin and epicatechin on the growth of the multiresistant strain C6840 is shown in Figure 2. MIC value of both polyphenols was 0.67 mg/mL. Moreover, 1.33 mg/mL malvidin showed bactericidal effect on this multidrug resistant strain, whereas epicatechin showed bacterostatic effect.

The grape polyphenol extract GG showed an inhibitory effect of bacterial growth of both multiresistant strains (Figures 3 & 4). The MIC of GG was 12.5 mg/ml (green lines) for both strains, and 6.25 mg/ml (yellow line) decreased bacterial population below 50% of the control (grey line). This effect was bacteriostatic, as bacterial growth was recovered in absence of the polyphenol extract.

**Synergy effect:** GG in combination with AMP showed synergistic activity (FIC= 0.4 and 0.6 for C6898 and C6840 respectively), and in the presence of 1.5 - 3.1 mg/mL of GG, bacterial growth was totally inhibited (red lines Figures 3 & 4). These results indicate that the presence of GG extract of Graciano grape skins turned both multiresistant *E. coli* strains sensitive to a small concentration of AMP.

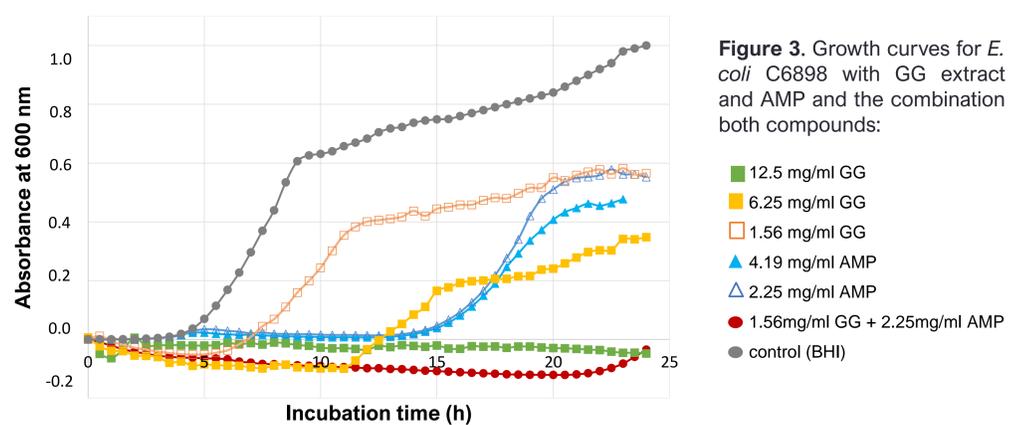


Figure 3. Growth curves for *E. coli* C6898 with GG extract and AMP and the combination both compounds:

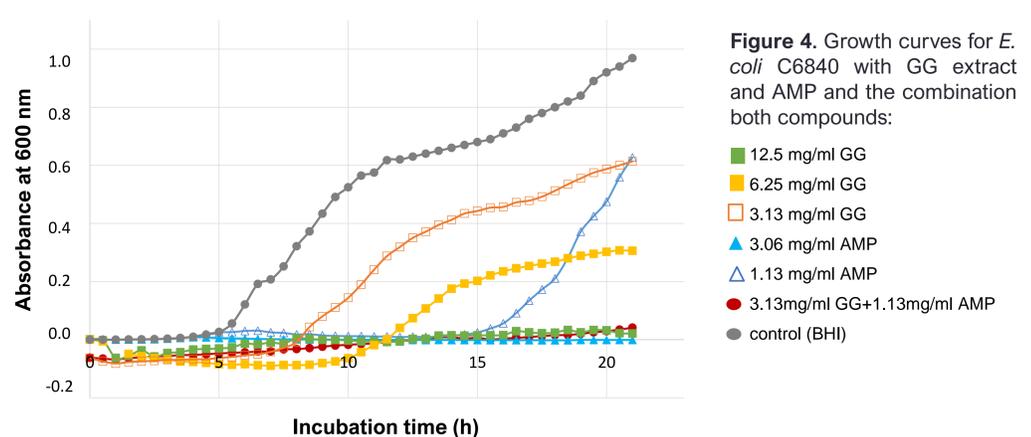


Figure 4. Growth curves for *E. coli* C6840 with GG extract and AMP and the combination both compounds:

## CONCLUSIONS

- A natural polyphenol extract obtained from Graciano grape skins can be used to control the growth of multiresistant *E. coli* strains.
- Grape skin polyphenol extracts could contribute to reduce the current prophylactic and therapeutic use of antibiotics, and thus contribute to combat widespread antibiotic resistance.

## REFERENCES

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