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Inserm

ADJUVANT EFFECT OF FARNESOL ENCAPSULATED WITHIN NANOCAPSULES ON COLISTIN EFFICACY AGAINST MCR-1 *E.coli*

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INTRODUCTION

The use of colistin, has been limited in the past because of its nephrotoxicity. Nowadays, colistin has become one of last antibiotic yet active against some multiresistant Gram negative bacteria. But, the discovery in 2015 of a transferable plasmid-encoded colistin resistance gene MCR-1 raised an important public health problem. Also, to keep this ATB as a last resort ATB a way to tackle this MCR-1 mechanism would be very useful.

Non-antibiotic drugs, like essential oils (EOs), combined to ATB have shown effectiveness against resistant bacteria. The aim of the study was to propose an effective combination between colistin and some EOs compounds against MCR-1 bacteria. Interesting results were obtained using geraniol and farnesol encapsulated within lipid nanocapsules (EO-LNCs).

MATERIAL & METHODS

Formulation LNCs

Lipophilic geraniol (LogP: 2.9) and farnesol (LogP: 4.84) were encapsulated within LNCs (20% w/w). LNCs were formulated using the phase inversion method, to facilitate the EOs aqueous dispersion. Three temperature cycles (60 - 90°C) were made to pass from a O/W emulsion to a W/O emulsion. At temperatures corresponding to the phase inversion zone (PIZ) LNCs were formed **(Fig 1a)**. LNCs were composed by a lipophilic core and a hydrophilic shell **(Fig 1b)**.

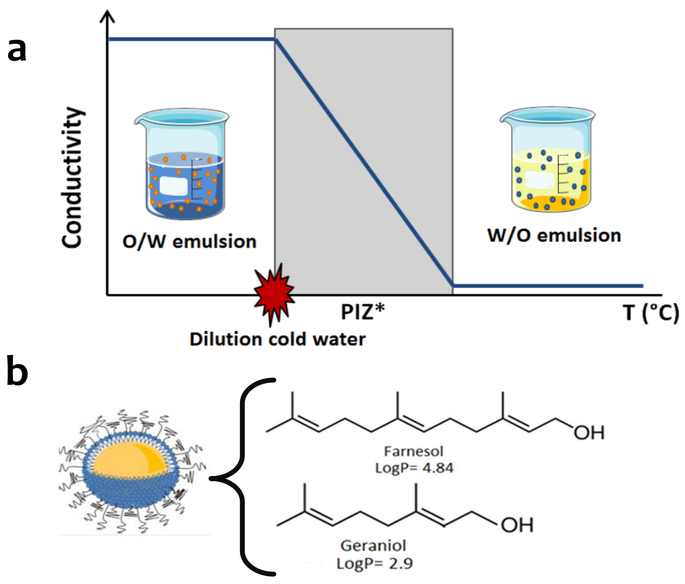

Fig.1: (a) Schematic representation of LNCs formulation. (b) Lipid nanocapsule loaded with farnesol or geraniol.

Diameters, polydispersity indexes (PDI) and zeta potentials of the LNCs were determined using a Zetasizer Nano ZS (Malvern).

Checkerboard titration

The diminution of colistin MIC in the presence of farnesol and geraniol was determined by checkerboard titration. **Potency (EC50)** and **Efficacy (Emax)** were determined for each combinations using an Emax model **(Eq.1)**.

$$\widehat{MIC} = MIC_0 - \frac{((MIC_0)-(MIC_\infty)* C_{EO}^{\gamma})}{EC50^{\gamma}+ C_{EO}^{\gamma}} \qquad Emax = \frac{MIC_0}{MIC_\infty}$$

Eq.1: Emax model equation.

Kill-time experiments

The time-kill studies were performed with an inoculum of 5.10^5 CFU/mL. Colistin concentration was 0.031 mg/L for *E.coli* J53 and 1mg/L for its MCR-1 transconjugant.

Based on checkerboard results, farnesol concentrations tested were; 10, 30 and 60 mg/L and geraniol concentrations were 60, 100 and 200 mg/L.

RESULTATS & DISCUSSION

LNCs characterisation

Reproductibles and monodisperses EO-LNCs (PDI= 0.03-0.59) were obtained . LNC were characterized by a mean diameter of 65-85 nm and a negative zeta potential (-0.5 to -2.2 mV) at pH7.

Emax and EC50 determination

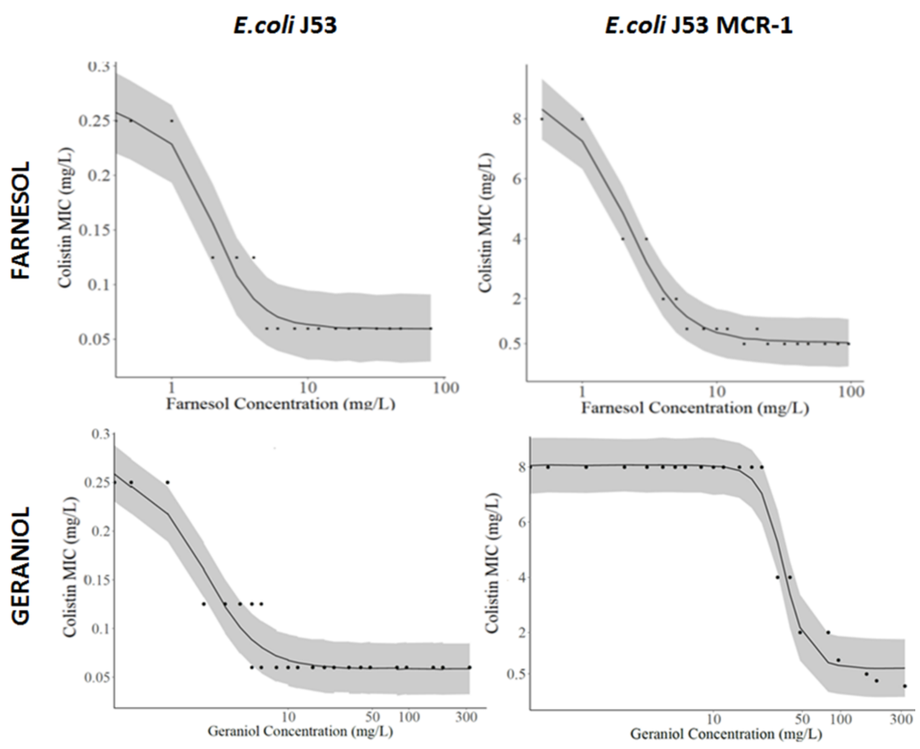

Fig.2: Relation between colistin MIC and EOs-LNC concentration, for the combination colistin/farnesol against *E.coli* J53 (a) and *E.coli* J53 MCR-1 (b), and for the combination colistin/geraniol against *E.coli* J53 (c) and *E.coli* J53 MCR-1 (d).

As geraniol and farnesol did not show any intrinsic antibacterial activity against these strains, the fractional inhibitory concentration (FIC) index could not be used to evaluate the effect of the colistin - EO-LNCs combination. Instead, an Emax model was used (Eq. 1).

Farnesol was more potent (Fig 2b) than geraniol against the MCR-1 strain to reduce the colistin MIC as described by the EC50 values (2.0 mg/L (± 0.24) for farnesol and 35.7 mg/L (± 2.03) for geraniol).

But **geraniol was more effective (Fig 2d)**, with a higher Emax (maximal effect) 66 for geraniol and 16 for farnesol.

Against sensitive strain, similar profiles were found with geraniol or farnesol **(Fig 2a, 2c)**.

Kill-time curves

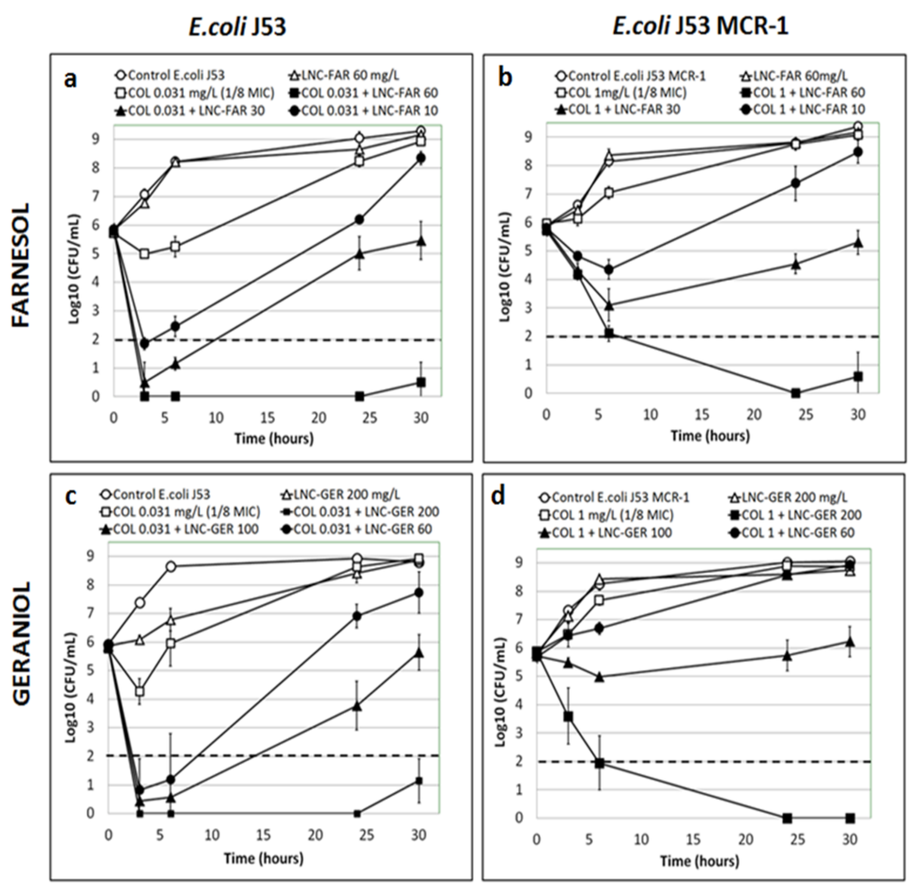

Fig.3: Time kill curves of EOs-LNC and colistin used alone and in combination. Farnesol and colistin against *E.coli* J53 (a), farnesol and colistin against *E.coli* J53_MCR-1 (b), geraniol and colistin against *E.coli* J53(c), geraniol and colistin against *E.coli* J53_MCR-1 (d).

Only the highest EOs concentration used for each combinations (60 mg/L for farnesol and 200 mg/L for geraniol) proved to completely prevent bacterial regrowth.

Against MCR-1 strain:

A bactericidal effect was observed for 60 mg/L of farnesol and 1 mg/L of colistin (1/8 of the MIC) after 6 hours of incubation, without bacterial regrowth for up to 30 hours after incubation **(Fig 3b)**.

Similar results were found with 200mg/L of geraniol and 1mg/L of colistin **(Fig 3d)**.

Against sensitive strain:

The bactericidal effect was observed for the combination farnesol 60 mg/L and colistin 0.031 mg/L, or geraniol 200 mg/L and colistin 0.031 mg/L after 3 hours of incubation **(Fig 3a, 3c)** without bacterial regrowth for up to 30 hours after incubation .

CONCLUSION

These adjuvants seem a good prospect for restoring colistin activity against MCR-1 *E.coli,* allowing using low colistin concentrations (1/8 of the MIC), while, preventing bacterial regrowth. The use of a molecule without bactericidal effect could slow down the appearance of resistances.

ACKNOWLEDGEMENTS

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