Magnetite nanoparticles functionalized with plant derived compounds reduce the resistance and persistence of opportunistic pathogens

[1,2,3]A.M. Holban*, [2]E. Andronescu, [2]A.M. Grumezescu, [1,3]L.M. Ditu, [1,3]C. Curutiu, [1]V. Lazar, [2,4]V. Grumezescu, Otilia Vasile[2], [1,3]C.M. Chifiriuc

[1]University of Bucharest, Bucharest, Romania, [2]University Politehnica of Bucharest, Bucharest, Romania, [3]Research Institute of the University of Bucharest, Bucharest, Romania, [4]INFLPR, Magurele, Romania; *corresponding author's e-mail: alina_m_h@yahoo.com

BACKGROUND: Nanosized shuttles developed for the transport, delivery and stabilization of antimicrobial agents represent efficient alternatives to balance the current antibiotics inefficiency. The aim of this study was to obtain antimicrobial magnetite nanoparticles functionalized with five plants derived natural compounds, efficient against biofilm embedded and planktonic *Pseudomonas aeruginosa* and *Staphylococcus aureus* cells.

OBSERVATIONS: Magnetite nanoparticles (Fe3O4@) have been synthesized by co-precipitation, characterized by IR, SEM and HR TEM and functionalized with eugenol (E), eucalyptol (e), carvone (C), limonene (L) and beta-pinene (BP). Minimum inhibitory concentrations were established by broth microdilution, while biofilm formation and production of persisters were assessed by viable counts. Our results demonstrated that all obtained nanoformulations inhibited bacterial multiplication, attachment and biofilm formation in a dose dependent manner, i.e.: Fe3O4@E>Fe3O4@L>Fe3O4@C>Fe3O4@BP>Fe3O4@e. Furthermore, subinhibitory concentrations of the obtained nanobioactive materials modulated the resistance of reference and clinical strains to Gentamicin (G) and Norfloxacin (N). Fe3O4@E significantly reduced the formation of persister cells in planktonic cultures of P. aeruginosa when grown in the presence of 10microgram/mL G ($p<0.05$) or 3microgram/mL N ($p<0.01$), and of S. aureus in the presence of 7microgram/mL G or 0.3microgram/mL N ($p<0.05$). Fe3O4@E and Fe3O4@Calso reduced the selection of resistant cells in P. aeruginosa and S. aureus monospecific static biofilms grown in the presence of G and N.

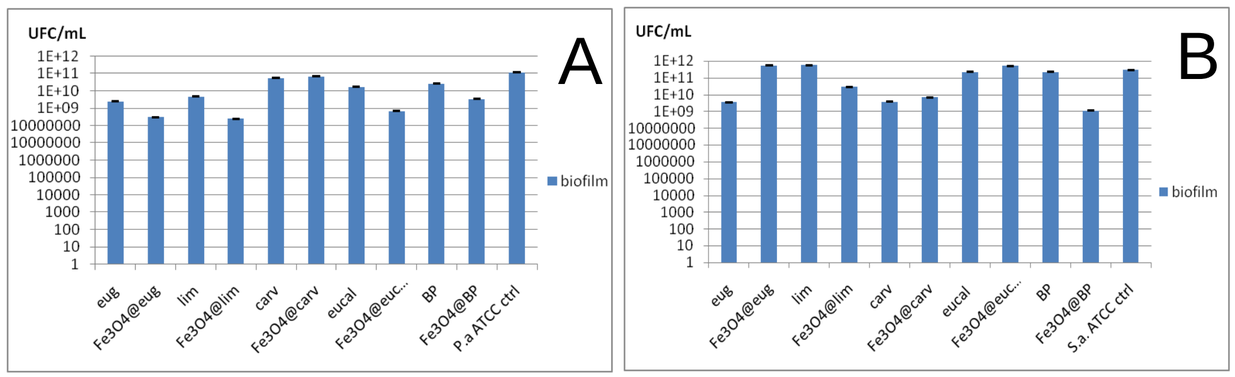

Fig. 3. Persister selection in *P. aeruginosa (A)* and *S. aureus (B)* biofilms grown in the presence of 20µg/mL gentamicin, represented as UFC/mL values.

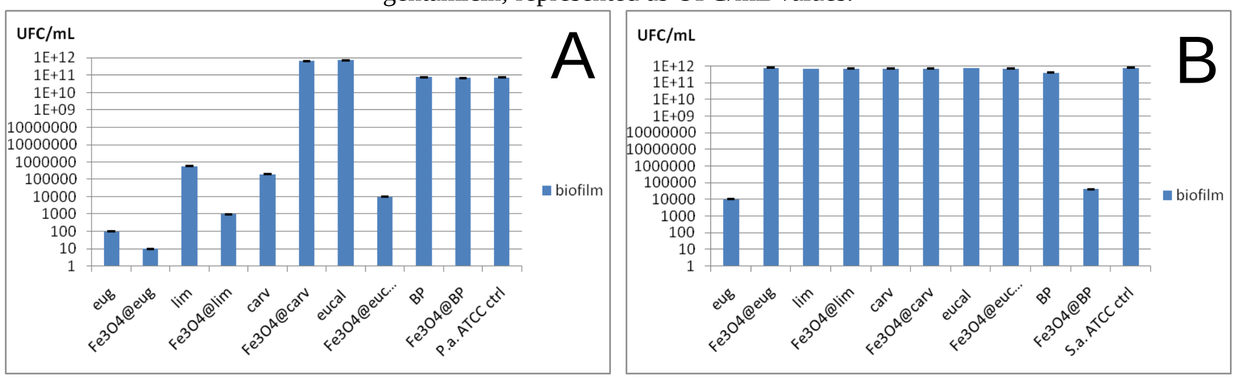

Fig. 4. Persister selection in *P. aeruginosa (A)* and *S. aureus (B)* biofilms grown in the presence of 3µg/mL norfloxacin, represented as UFC/mL values.

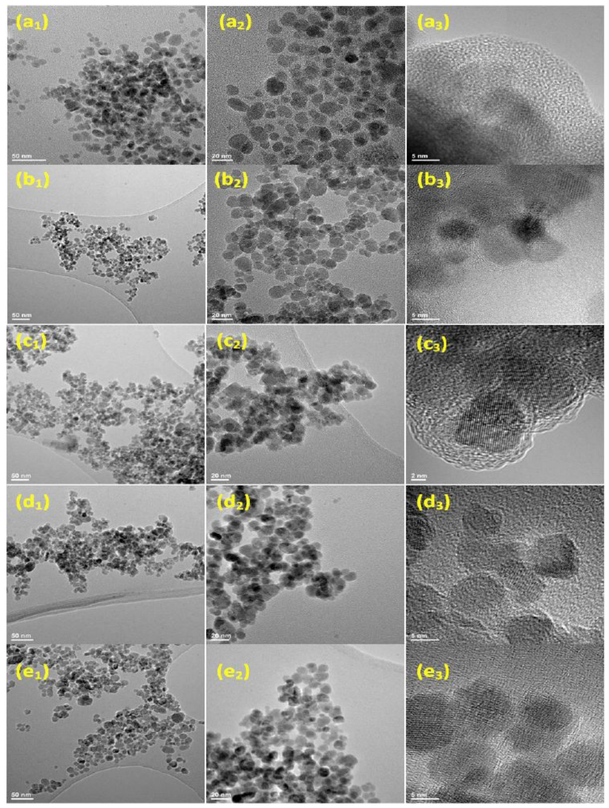

Fig. 2. TEM (1,2) and HR-TEM (3) images for the obtained nanosystems (a) Fe_3O_4@BPIN; (b) Fe_3O_4@CAR; (c) Fe_3O_4@EUC; (d) Fe_3O_4@EUG; (e) Fe_3O_4@LIM;

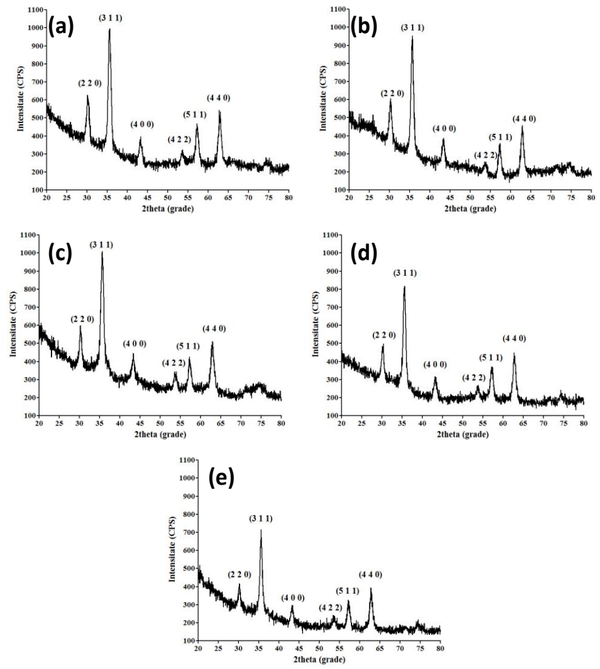

Fig. 1. X-ray diffractograms for (a) Fe_3O_4@BPIN; (b) Fe_3O_4@CAR; (c) Fe_3O_4@EUC; (d) Fe_3O_4@EUG; (e) Fe_3O_4@LIM;

CONCLUSIONS: The obtained data demonstrate that magnetite nanoparticles functionalized with plant-derived compounds could represent successful combinations for the development of efficient antimicrobial strategies based on natural products to fight severe infections produced by resistant pathogens.

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